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October 1991 in two parts, part 1 volume 165, number 4

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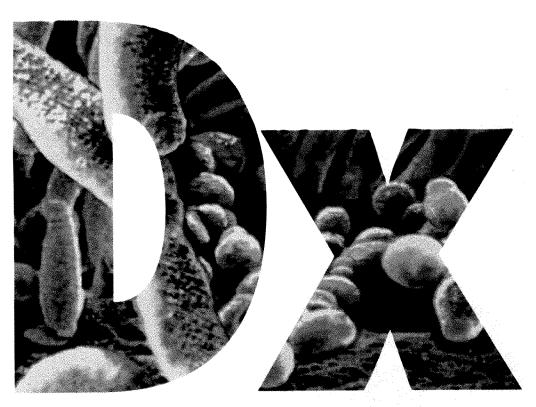
AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY SOCIETY FOR GYNECOLOGIC INVESTIGATION SOCIETY OF PERINATAL OBSTETRICIANS



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Artist's enhancement of scanning electron microscopy: C albicans 24 hours posttreatment with butoconazole nitrate.*

When you diagnose vulvovaginal candidiasis (VVC)

FEMSTAT® Prefill (butoconazole nitrate) Vaginal Cream 2% is clinically effective against a broad spectrum of important pathogens, including *Candida albicans* and other *Candida* species, such as *C tropicalis*.

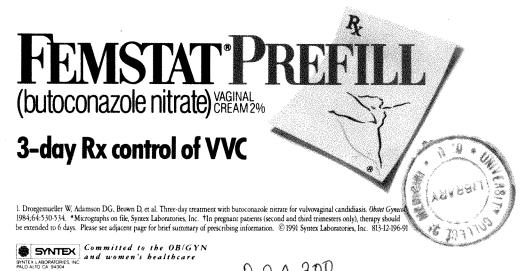


Artist's enhancement of scanning electron microscopy: C albicans one week posttreatment with butoconazole nitrate.*



Prescribe the efficacy of 3-day therapy⁺

FEMSTAT achieves a microbiological cure rate of 95% with the original 3-day therapy¹ that is not confirmed to be associated with flu-like symptoms. The most common complaint with the use of FEMSTAT in clinical trials was vulvar/vaginal burning (2.3%).



P 24,300



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS. Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION

Federal law prohibits dispensing without prescription.



YOUR PATIENTS COUNT ON YOU FOR ANSWERS...

Here are two "must-have" resources to help you explain the mysteries of childbirth to your patients. Using dramatic full-color photos and ultrasound scans, these atlases provide step-by-step coverage of fetal development and childbirth.

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A COLOR ATLAS OF CHILDBIRTH AND OBSTETRIC TECHNIQUES

Farook Al-Azzawi, MBChB, MA, PhD, MRCOG

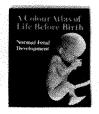
- Includes coverage of breech delivery, management of twins, operative vaginal delivery and the three most common types of caesarean births.
- Full color photographs depict all aspects of childbirth, with line drawings used to explain movement of the baby.
- Covers prenatal care and management of labor.
 1991. 144 pages, 341 ills. A Wolfe Medical Publishers, Ltd. title. (Book Code: 06287)

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COLOR ATLAS OF LIFE BEFORE BIRTH: Normal Fetal Development

Marjorie A. England

- Section I covers overall development of the embryo and fetus and their relationship to the placenta, amnion and chorionic sac.
- Section II provides extensive details on the development of individual organ systems.



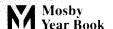
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October Part 1

This issue contains Transactions of the Eleventh Annual Meeting of the Society of Perinatal Obstetricians and a Special Report, The Pathology of Maternal Mortality, beginning on page 1126.

TRANSACTIONS OF THE ELEVENTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

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Ran Neiger, MD, and Donald R. Coustan, MD Providence, Rhode Island

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Enhanced endothelium-derived relaxing factor activity in pregnant, spontaneously hypertensive rats

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Robert A. Ahokas, PhD, Brian M. Mercer, MD, and Baha M. Sibai, MD Memphis, Tennessee

Increased basal endothelium-derived relaxing factor activity may be responsible for pregnancy vasodilation and the fall in blood pressure in spontaneously hypertensive rats.

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New Haven, Connecticut

Amniotic fluid white blood cell count is a rapid, sensitive, and inexpensive method for the diagnosis of microbial invasion of the amniotic cavity and is a specific method for detection of the patient at risk for preterm delivery.

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single-dose prophylaxis/ twice-daily treatment



CEFOTAN[®]
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: 1. Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefowitin usage to cefotetam: an interdisciplinary approach. Am J Surg. 1988, 155(5A):101-102. 2. Sochalski A, Sullman S, Androile VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. Am J Surg. 1988;155(5A):96-101. 3. CEFOTAN® (cefotetan dissodium) full prescribing information issued March. 1986. 4. Physicians' Desk Reference. ed 43. Oradell, NJ. Medical Economics. Co. 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. 5. Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. Infect. Surg. April 1986 (suppl), pp 11-14. 6. Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. Am J Surg. 1988;155(5A):67-70. 7. Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. MMWR. 1989;38(suppl S-8):1-43.

CEFOTAN (cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use (FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.)

INDICATIONS AND USAGE
Treatment: CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary fract infections caused by Ecoli. Klebsiella species (including K pneumoniae), Proteus mirabilis, and Proteus sp (which may include the organisms now called Proteus vulgaris, Providencia rettgeri, and Morganella morganii).
Lower Respiratory Tract Infections caused by Streptococcus pneumoniae (formerly D pneumoniae), Staphylococcus aureus (penicillinase- and nonpenicillinase-producing strains), Haemophilus influenzae (including ampicillin-resistant strains), Klebsiella species (including K pneumoniae), E coli, Proteus mirabilis and Serratia marcescens:

Lower Respiratory Tract Infections caused by Streptococcus pneumoniae (formerly D pneumoniae), Staphylococcus aureus (penicillinase- and nonpenicillinase-producing strains). Haemophilus influenzae (including ampicillin-resistant strains), Klebsiella species (including K pneumoniae), E coli, Proteus mirabilis, and Serratia marcescens:

Skin and Skin Structure Infections caused by Staphylococcus aureus (penicillinase- and nonpenicillinase-producing strains). Staphylococcus epidermidis, Streptococcus progenes, Streptococcus species (excluding enterococci), E coli, Klebsiella pneumoniae, and Peptoscus' and Peptostreptococcus so', Synecologic Infections caused by Staphylococcus aureus (including penicillinase- and nonpenicillinase-producing strains), Staphylococcus epidermidis, Streptococcus species (excluding enterococci), So producing strains), Staphylococcus epidermidis, Streptococcus species (excluding B distasonis, B ovatus, and B thetalotaomicron), Fusobacterium species,* and gram-positive anaerobic cocci (including Peptococcus and Peptostreptococcus species) (excluding Peptococcus and Peptostreptococcus species) (excluding B distasonis, B ovatus, and B thetalotaomicron), and Clostridium species.*

Bone and Joint Infections caused by Staphylococcus aureus.*

Efficacy for this organism in this organ system was studied in tever than ten infections. Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known, however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vi

OCEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics. WARNINGS

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. It an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures. Pseudomembranous colitis has been reported with the use of cephalosporins (and other broadspectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.
Treatment with broad-spectrum antibiotics may after normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by Clostridium difficile is one primary cause of antibiotic-associated colitis.
Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be

antibiotic associated colitis.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by C difficile. Other causes should also be considered. In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated. PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

CEFOTAN should be used with caution in individuals with a history of gastrointestanal disease, particularly coditis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminorlycoside.

alone, it is possible that hephrotoxicity may be potentiated in our other is used concomining which an aminoglycoside. **Drugf_aboratory Test Interactions:** A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported. **Carcinogenesis, Mutagenesis, impairment of Fertility:** Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

been permitted to evaluate satisfied by the permitted permitted by the per

500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methythiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day I/for 5 weeks. The relevance of these finidings to humans is unknown.

Usage in Prepnanery: Prepnanery Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to ceftotelan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when ceftotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

tastromasmas symptoms occurred in 1.5% of patients, the most nequent were diarriae (1 in 50) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300). Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).

(1 in 300), alkaline prosponatase (1 in rou), and 10 patients and included rash (1 in 100) and including thypersensitivity reactions were reported in 1.2% of patients and included phlebitis at the site of injection (1 in 300).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300).

During postmarketing experience with CEFOTAN, agranulocytosis, anaphylactic reactions, fever, hermolytic anemia, leukopenia, prolonged prothrombin time with or without bleeding, pseudomembranous colitis, and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

Treatment: The usual addut dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN				
Type of Infection Daily Dose Frequency and Route			Type of Infection	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM		
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM		
Severe	4 grams	2 g every 12 hours IV		
Life-Threatening	6* grams	3 g every 12 hours IV		

*Maximum daily dosage should not exceed 6 grams

Prophylaxis: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umblical cord is clamped.

Impaired Renal Function: When renal function is impaired, a reduced dosage schedule must be

employed. The following dosage guidelines may be used.

Creatinine Clearance	DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION	
mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
~10	Usual Recommended Dosane*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

Weight (kg) × (140 - age) Males: 72 × serum creatinine (mg/100 mL)

> Females: 0.9 x value for males

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

Manufactured for



Rev. S 05/91

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Shoulder dystocia: Should the fetus weighing ≥4000 grams be delivered 831 by cesarean section? Oded Langer, MD, Michael D. Berkus, MD, Robert W. Huff, MD, and Arnon Samueloff, MD San Antonio, Texas In diabetic women with infant birth weights of ≥4250 gm, 80% of cases of shoulder dystocia will result, whereas only 20% of cases will be identified in the nondiabetic cafegory. A five-year statewide experience with congenital diaphragmatic hernia 838 Katharine D. Wenstrom, MD, Carl P. Weiner, MD, and James W. Hanson, MD Iowa City, Iowa A 5-year statewide survey indicates that 55% of infants with isolated congenital diaphragmatic hernia survive without in utero surgery. Intrapartum ultrasonographic estimates of fetal weight by the house staff 842 Deborah N. Platek, MD, Michael Y. Divon, MD, Akolisa Anyaegbunam, MD, and Irwin R. Merkatz, MD Bronx. New York Ultrasonographic estimates of fetal weight performed by residents in a busy inner-city labor and delivery ward were evaluated. The cheek-to-cheek diameter in the ultrasonographic assessment of fetal 846 growth Jacques S. Abramowicz, MD, David M. Sherer, MD, Eli Bar-Tov, PhD, and James R. Woods, Jr., MD Rochester, New York Prospective ultrasonography of 200 appropriate-for-gestational-age fetuses showed that the cheek-to-cheek measurement correlated with and the cheek-to-cheek diameter/biparietal diameter ratio was independent of gestational age between 20 and 41 weeks. 853 Transforming growth factor- β_1 expression during placental development Lauren J. Dungy, MD, Tariq A. Siddiqi, MD, and Sohaib Khan, PhD Cincinnati, Ohio Peak expression of transforming growth factor- β_1 in human placenta near mid and late gestation may be related to inhibition of cytotrophoblastic proliferation. Multicenter randomized clinical trial of home uterine activity monitoring 858 for detection of preterm labor Susan M. Mou, MD, Shirazali G. Sunderji, MD, Stanley Gall, MD, Helen How, MD, Vinu Patel, MD, Mark Gray, MD, Herbert L. Kayne, PhD, and Michael Corwin, MD Kansas City, Missouri, Syracuse, New York, Chicago, Illinois, and Boston, Massachusetts Home uterine activity monitoring in addition to obstetric care in women at risk for preterm labor detects labor at lesser cervical dilatation, allowing prolongation of pregnancy and improved perinatal outcome.

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James A. McGregor, MDCM, Janice I. French, CNM, MS, and Kyung Seo, MD, PhD Denver, Colorado

A defined course of clindamycin was associated with prolongation of pregnancy in women treated for idiopathic labor before 33 weeks' gestation.

Fetal cardiac function in intrauterine growth retardation

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Giuseppe Rizzo, MD, and Domenico Arduini, MD Rome, Italy

Several abnormalities of cardiac function are present in fetuses with intrauterine growth retardation. Longitudinal monitoring from diagnosis to the onset of antepartum late heart rate decelerations showed a progressive fall in cardiac output and in pulmonary and aortic peak velocities.

When do cardiovascular parameters return to their preconception values?

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Eleanor J. Capeless, MD, and James F. Clapp, MD Burlington, Vermont

Stroke volume and end-diastolic volume remain elevated over their preconceptional baseline values in a population of breast-feeding women.

Baroreflex function in normal pregnancy

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Line Leduc, MD, Nathan Wasserstrum, PhD, MD, Thomas Spillman, PhD, and David B. Cotton, MD Houston, Texas

Baroreflex sensitivity was increased in normal pregnancy at term; the attenuated pressor response to phenylephrine may result in part from increased baroreflex sensitivity.

Changes in immunologic parameters in normal pregnancy and spontaneous abortion

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Marjory A. MacLean, MB, ChB, Rhoda Wilson, PhD, John A. Thomson, MD, Srinivasan Krishnamurthy, MB, ChB, and James J. Walker, MB, ChB Glasgow, Scotland

Immunologic changes occurring in normal pregnancy suggest an increased potential to respond to an immunologic challenge; in spontaneous abortion there is evidence of immunologic activation.

High plasma cellular fibronectin levels correlate with biochemical and clinical features of preeclampsia but cannot be attributed to hypertension alone

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Robert N. Taylor, MD, PhD, William R. Crombleholme, MD, Steven A. Friedman, MD, Lynn A. Jones, MA, David C. Casal, PhD, and James M. Roberts, MD San Francisco and Sunnyvale, California

Plasma cellular fibronectin concentrations were found to be significantly higher in women with preeclampsia relative to levels in matched women with normal pregnancy and pregnant women with transient hypertension.

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The programmable external infusion pump appears to be an effective, safe method of administering heparin for indications during pregnancy.

Placental pathologic findings in preterm birth

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C.M. Salafia, MD, C.A. Vogel, MD, A.M. Vintzileos, MD, K.F. Bantham, MD, J. Pezzullo, PhD, and L. Silberman, MD

Danbury and Farmington, Connecticut, and Providence, Rhode Island

Decidual vascular abnormality, umbilical-chorionic vasculitis, and chronic villitis were associated with preterm birth.

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Robert L. Jacobson, MD, Anthony Brewer, BS, Annie Eis, MS, Tariq A. Siddiqi, MD, and Leslie Myatt, PhD Cincinnati, Ohio

Low-dose aspirin rapidly crosses the placenta but does not affect perfusion pressure.

Report of fourteen cases of nonimmune hydrops fetalis in association with hemorrhagic endovasculitis of the placenta

945

Patricia M. Novak, DO, C. Maureen Sander, MD, S. Samuel Yang, MD, and Paul T. von Oeyen, MD Royal Oak, East Lansing, and Detroit, Michigan

Fourteen cases of nonimmune hydrops fetalis were found in connection with hemorrhagic endovasculitis; in eight of them the placental abnormality was the only associated significant pathologic condition.

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John R. Barton, MD, Rebecca R. Prevost, PharmD, Donald A. Wilson, MD, W. David Whybrew, MS, and Baha M. Sibai, MD

Memphis, Tennessee

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Seattle, Washington

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Do catechol estrogens participate in the initiation of labor?

984

Anjan Biswas, PhD, Anjan Chaudhury, MD, Sati C. Chattoraj, PhD, and Sidney L. Dale, PhD Boston, Massachusetts

Catechol estrogens in amniotic fluid increase as pregnancy progresses and are significantly higher in spontaneous labor at term in comparison with cesarean section not in labor.

The effect of fetal movement counting on maternal attachment to fetus

988

Magdy S. Mikhail, MD, Margaret C. Freda, EdD, RN, Ruth B. Merkatz, PhD, RN, Regina Polizzotto, MS, CNM, Evelyn Mazloom, MPH, CNM, and Irwin R. Merkatz, MD Bronx, New York

Women who counted their fetal movements had higher maternal-fetal attachment scores, as compared with those of controls.

A randomized, double-blind trial of prostaglandin E₂ gel for cervical ripening and meta-analysis

991

John Owen, MD, Carey L. Winkler, MD, Bruce A. Harris, Jr., MD, John C. Hauth, MD, and Mary C. Smith, PhD

Birmingham, Alabama

Both a double-blind trial and a meta-analysis of prostaglandin E_2 gel for cervical ripening showed no benefit with regard to cesarean section delivery rate.

Uterine rupture during trial of labor after previous cesarean section

996

Richard M. Farmer, MD, PhD, Thomas Kirschbaum, MD, Daniel Potter, Thomas H. Strong, MD, and Arnold L. Medearis, MD

Los Angeles, California

The Los Angeles County/University of Southern California Women's Hospital experience with uterine rupture in patients undergoing a trial of labor after a previous cesarean section from 1983 to 1989 was reviewed, with incidence rates, associations, and recommendations made.

Evaluation of obstetric ultrasonography at the first prenatal visit

1002

Jeffrey M. Barrett, MD, and Jennifer Brinson, RNC Lakeland, Florida

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Patterns of uterine activity after intravaginal prostaglandin E_2 during preinduction cervical ripening

1006

Ann M. Miller, BSN, William F. Rayburn, MD, and Carl V. Smith, MD Omaha, Nebraska

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"Meconiumcrit" and birth asphyxia

1010

Kenneth J. Trimmer, MD, and Larry C. Gilstrap III, MD Dallas, Texas

The data from this investigation indicate that there is no correlation between "thickness" of meconium and recently reported markers of birth asphyxia.

The relationship between umbilical artery Doppler velocimetry and fetal biometry

1013

William E. Scorza, MD, Deborah Nardi, RT(R), RDMS, Anthony M. Vintzileos, MD, Alfred D. Fleming, MD, John F. Rodis, MD, and Winston A. Campbell, MD

Farmington, Connecticut

There is correlation between umbilical artery peak-systolic/end-diastolic ratio and fetal biometry; nomograms between peak-systolic/end-diastolic ratio and fetal biometric parameters can be used in patients with unknown or uncertain dates of gestation.

Risk factors for cordocentesis and fetal intravascular transfusion

1020

Carl P. Weiner, MD, Katharine D. Wenstrom, MD, Susan L. Sipes, MD, and Roger A. Williamson, MD Iowa City, Iowa

The risk of accessing the fetal umbilical circulation reflects both the indication and the vessel punctured.

Fetal blood sampling in patients undergoing elective cesarean section: A correlation with cord blood gas values obtained at delivery

1026

Aldo D. Khoury, MD, Michael L. Moretti, MD, John R. Barton, MD, David C. Shaver, MD, and Baha M. Sibai, MD

Memphis, Tennessee

Blood gas parameters obtained by cordocentesis were found to be significantly different from those in cord blood samples obtained at delivery in 18 patients with uncomplicated pregnancies undergoing elective cesarean section at term.

Amniotic fluid bilirubin and fetal hemolytic disease

1030

Joseph A. Spinnato, MD, Kathleen K. Ralston, MT (ASCP), Eileen R. Greenwell, MT (ASCP), Carolyn A. Marcell, RN, and Joseph A. Spinnato III, BS Louisville, Kentucky

Chloroform-extracted amniotic fluid ΔOD_{450} accurately predicts the severity of fetal hemolytic disease; its use should continue.

What is a low-lying placenta?

1036

Lawrence W. Oppenheimer, MD, Dan Farine, MD, J.W. Knox Ritchie, MD, Reuven M. Lewinsky, MD, Joyce Telford, RN, and Lea A. Fairbanks, MD

Toronto, Ontario, Canada

Criteria for diagnosing low-lying placenta with transvaginal ultrasonography are outlined, and their value with respect to management and prognosis is described.

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Pulmonary-to-aorta diameter ratio in the normal and abnormal fetal heart

1038

Christine H. Comstock, MD, Thomas Riggs, MD, Wesley Lee, MD, and Janet Kirk, MD Royal Oak, Michigan

The ratio of the diameters of the fetal pulmonary artery to the aorta is independent of gestational age.

Transverse cerebellar diameter: A useful predictor of gestational age for fetuses with asymmetric growth retardation

1044

Wesley Lee, MD, Scott Barton, MD, Christine H. Comstock, MD, Stephanie Bajorek, RDMS, Daniel Batton, MD, and Janet S. Kirk, MD Royal Oak, Michigan

Gestational age in growth-retarded fetuses is estimated best by transverse cerebellar diameter when asymmetry between the head and abdomen is present.

Comparison of humerus length with femur length in fetuses with Down syndrome

1051

John F. Rodis, MD, Anthony M. Vintzileos, MD, Alfred D. Fleming, MD, Leslie Ciarleglio, MS, Deborah A. Nardi, RRT, RDMS, Lori Feeney, RRT, RDMS, William E. Scorza, MD, Winston A. Campbell, MD, and Charles Ingardia, MD

Farmington and Hartford, Connecticut

Second-trimester fetal humerus length was abnormally short (<5%) in 64% of cases of Down syndrome diagnosed prenatally; an abnormally short fetal humerus should prompt genetic studies (e.g., amniocentesis).

Ultrasonographic diagnosis of congenital anomalies in twins

1056

Steven R. Allen, MD, Leslie J. Gray, MD, Barbara H. Frentzen, MSN, and Amelia C. Cruz, MD Gainesville, Florida

Prenatal ultrasonography among twins diagnosed 69% of the major anomalies leading to altered perinatal management; the four-chamber view was an inadequate cardiac screen.

Does amniotic fluid index affect the accuracy of estimated fetal weight in preterm premature rupture of membranes?

1060

Julianne S. Toohey, MD, David F. Lewis, MD, James A. Harding, MD, Michael Crade, MD, Tamerou Asrat, MD, Carol A. Major, MD, Thomas J. Garite, MD, and Manuel Porto, MD Orange and Long Beach, California

Ultrasonography-estimated fetal weight in patients with preterm premature rupture of membranes appears to be accurate and independent of amniotic fluid volume.

Transcervical chorionic villus sampling and midtrimester oligohydramnios

1063

Edith Y. Cheng, MD, David A. Luthy, MD, Durlin E. Hickok, MD, Kathryn A. Hollenbach, PhD, Robert G. Resta, MS, Barry S. Mahony, MD, and Frederick W. Luthardt, PhD Seattle, Washington

First-trimester transcervical chorionic villus sampling was associated with a significantly increased risk for midtrimester oligohydramnios and its associated poor prognosis, as compared with midtrimester amniocentesis.

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Intrapartum course of fetuses with isolated hypoplastic left heart syndrome

1068

G. Marc Jackson, MD, Jack Ludmir, MD, Arthur J. Castelbaum, MD, James C. Huhta, MD, and Arnold W. Cohen, MD

Philadelphia, Pennsylvania

Labor and delivery does not appear to be a high-risk situation for the fetus with hypoplastic left heart syndrome who is free of other abnormalities.

Fetal movement during labor

1073

Uma M. Reddy, MD, Lisa L. Paine, CNM, DrPH, Carolyn L. Gegor, CNM, MS, Mary Jo Johnson, MD, and Timothy R.B. Johnson, MD

Baltimore, Maryland, and Providence, Rhode Island

This study demonstrates the high association between fetal movement and contractions during labor.

Oligohydramnios: Antepartum fetal urine production and intrapartum fetal distress

1077

Lynn J. Groome, PhD, MD, John Owen, MD, Cherry L. Neely, RT, RDMS, and John C. Hauth, MD Birmingham, Alabama

Women with oligohydramnios whose infants are delivered abdominally because of fetal distress in labor have significantly lower antepartum rates of fetal urine production than do women whose fetuses have no distress in labor.

Antepartum fetal surveillance tests during sickle cell crisis

1081

Akolisa Anyaegbunam, MD, Marie-Ignace Gauthier Morel, MD, and Irwin R. Merkatz, MD Bronx, New York

Although sickle cell disease crisis is associated with a higher incidence of abnormal biophysical test results, in most patients these results revert to normal after crisis.

Maternal perception of decreased fetal movement as an indication for antepartum testing in a low-risk population

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Janice E. Whitty, MD, David A. Garfinkel, MD, and Michael Y. Divon, MD Bronx, New York

Fetal surveillance is indicated in low-risk patients with complaints of decreased fetal movement; additional testing of patients with normal initial evaluation may not be necessary.

Correlation of amniotic fluid index and nonstress test in patients with preterm premature rupture of membranes

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James A. Harding, MD, David M. Jackson, MD, David F. Lewis, MD, Carol A. Major, MD, Michael P. Nageotte, MD, and Tamerou Asrat, MD *Orange and Long Beach, California*

Nonstress test observations in the patient with preterm premature rupture of membranes can help identify clinically reduced amniotic fluid volumes.

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Iffath A. Hoskins, MD, Faith J. Frieden, MD, and Bruce K. Young, MD New York, New York

Spontaneous variable decelerations in reactive nonstress tests coupled with oligohydramnios (amniotic fluid index <5) identify fetuses at high risk for compromise.

Preterm premature rupture of membranes: Detection of infection

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Periclis Roussis, MD, Richard L. Rosemond, MD, Cheryl Glass, RN, BSN, and Frank H. Boehm, MD Nashville, Tennessee

A suggested protocol involving daily biophysical profile monitoring is proposed for the expectant management of patients with preterm premature rupture of the membranes.

Correlation of amniotic fluid glucose concentration and intraamniotic infection in patients with preterm labor or premature rupture of membranes

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Daniel W. Gauthier, MD, William J. Meyer, MD, and Andre Bieniarz, MD Chicago, Illinois

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T. Asrat, MD, D.F. Lewis, MD, T.J. Garite, MD, C.A. Major, MD, M.P. Nageotte, MD, C.V. Towers, MD, D.M. Montgomery, MD, and W.A. Dorchester, PhD Orange and Long Beach, California

A retrospective analysis of 121 patients with 255 consecutive pregnancies complicated by preterm premature rupture of membranes is presented to define the rate of recurrence.

Renal pelvicalyceal dilation in antepartum pyelonephritis: Ultrasonographic findings

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Diane Twickler, MD, Bertis B. Little, PhD, Andrew J. Satin, MD, and Charles E.L. Brown, MD Dallas, Texas

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Norman B. Duerbeck, MD, Marcello Pietrantoni, MD, Kathryn L. Reed, MD, Caroline F. Anderson, RDMS, and Lewis Shenker, MD

Tucson, Arizona

Doppler flow velocity ratios in single umbilical arteries were normal in 77% of cases.

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N. Caccia, BSc, J.M. Johnson, MD, G.E. Robinson, MD, and T. Barna, BSc Toronto, Ontario, Canada

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Memphis, Tennessee, Los Angeles, California, and Boston, Massachusetts

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She needs PREMARIN to help prevent further bone loss

Calcium¹ and exercise are not enough to prevent postmenopausal osteoporosis, since estrogen deficiency is the primary cause.² PREMARIN is the only brand of estrogen indicated to prevent further bone loss and in a recently published study has been shown to reduce the risk of hip fractures by as much as 66%.³

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L ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

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There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

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INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism. castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and

PREMARIN (conjugated estrogens) vaginal cream is murcated in the treatment of allogated Ratiosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETTUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected strogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected strogen-dependent appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients. WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher than the property of the part of t

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women laking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of strogens has been reported.

Large doses of strogens such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myboardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose. Blood pressure should be monitored with estrogen use, especially if high doses are used. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should

include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T₄ levels determined by column or by radioimmunoassay. Free T₃ resin uptake is decreased, reflecting the elevated TBG; free T₄ concentration is unaftered.
3. Impaired discose hierance

Impaired glucose tolerance.
Reduced response to metyrapone test.
Reduced serum folale concentration

Reduced serim rotate concentration.
 MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See CONTRAINDICATIONS and Pened Mexicology.

and Boxed Warning
NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done
only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding
pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine
fibromyomata, vaginal candidiasis, change in amount of cervical secretion; lenderness or enlargement of breasts,
nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when
drug is discontinued, erythema multiflorme, erythema nodosum, hemorrhagic eruption, loss of salp hair,
hirsulism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental
depression, chorea, increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria;
adema: changes in libido.

depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema, changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN* Brand of conjugated estrogens tablets, USP

1. Given cyclically for short-term use only. For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg. three weeks on and one week off). Attempts to discontinue or taper medication should be made at three-to six-month intervals.

2. Given cyclically: Hypoestrogenism. Osteoporosis. Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. It bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg. three weeks on and one week off).

PREMARIN* Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-lerm use only. For Irealment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Aftempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact ulerus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

References:

1. Nilas L, Christiansen C, Bøddro P, Calcium supplementation and postmenopausal bone loss. *Br Med J* 1984, 289:1103-1106. 2, Riggs BL, Research Directions in Osteoporosis. Scientific Workshop, National Institutes of Health. February 9-11, 1987. 3, Kiel DP, Felson DT, Anderson JJ, et al. Hip tracture and the use of estrogens in postmenopausal women. *N Engl. J. Med.* 1987, 317. 1169–1174. 4, Christiansen C. Christensen MS, Transbel 1. Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lancet* 1981, 1459-461.





American Journal of Obstetrics and Gynecology

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With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

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Indications and Usage —TRIPHASIL.** is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications— OCs should not be used in women with any of the following: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Endometrial carcinoma or other known or suspected of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected or pregnancy. Known or suspected pregnancy.

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contra-ceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

Should be strongly advised not to smoke.

Use of OCs is associated with increased risks of serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although risk of serious morbidity/mortality is very small in healthy women without underlying risk factors. Morbidity/mortality risk increases significantly if other risk factors present (i.e. hypertension, hyperligidemias, obesity, diabetes). Practitioners prescribing OCs should be familiar with the following information relating to these risks. (This information is based principally on data involving OCs with higher doses of estrogen and progestogen than those commonly used today. Effect of long-term use of lower estrogen and progestogen formulations is yet to be determined.)

1. Thromboembolic Discorders and Other Vascular Problems— MyOCARDIAL INFARCTION (M), An increased risk of MI has been attributed to OC use. Risk is primarily in smokers or women with other underlying risk factors for coronary-aftery disease. (i.e. hypertension, hypercholesterolemia, morbid obesity, diabetes). Relative risk of heart attack for current OC users is estimated to be two to six; risk is very low under the age of 30. Smoking accounting for majority of excess cases. Mortality rates associated with circulatory disease increase substantially in smokers over the age of 35 and nonsmokers over the age of 40 among OC users.

OCs may compound effects of well-known risk factors, such as hypertension, diabetes, hyperligidemias, age and obesity, in particular, some progestogens decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. OCs have been shown to increase dood pressure among users (see Warnings). Similar effects on risk factors are associated with increased risk of heart disease. Use OCs with caution in women with cardiovascular disease risk factors.

THROMBOEMBOLISM. Increased risk of thromboembolic and thrombotic disease associated with OC use is wel

caution in women with cardiovascular disease risk factors.

THROMBOEMBOLMS L. Increased risk of thromboembolic and thrombotic disease associated with OC use is well established. In case control studies relative risk of users compared to non-users was 3 for first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1,5 to 6 for women with predisposing conditions for venous thromboembolic disease. In cohort studies relative risk was somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. Thromboembolic disease risk duer, about 3 for new cases and about 4.5 for new cases requiring hospitalization. Thromboembolic disease risk duer, about 4.6 for every cases requiring hospitalization. Thromboembolic disease risk duer, about 4.6 for every cases requiring hospitalization. Thromboembolic disease risk duer, and the relative risk of venous thrombosis in women with predisposing conditions is twice that of women without such conditions. If feasible, discontinue OCs at least 4 weeks prior to and for 2 weeks after elective surgery of a type associated with increased risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is associated with an increased thromboembolic risk, start OCs no earlier than 4 to 6 weeks after delivery in women not breast-feeding, or a mid-trimester pregnancy termination. CEREBROVASCULAR DISEASES. OCs increase relative and attributable risks of cerebrovascular events (thrombotic stokes); in general, risk is gratest among older / 58 years), hypertensive women (thrombotic and hemorrhagic strokes), in general, risk is greatest among older (> 35 years), hypertensive women who smoke. Hypertension is a risk factor for users and nonusers, for both types of strokes, while smoking interacts to increase hemorrhagic stoke risk. DOSE-RELATED RISK OF VASCULAR DISEASE FROM OCS. A positive association has been observed between

amount of estrogen and progestogen in OCs and vascular disease risk. A decline in serum high density lipoproteins (HDL) is reported with many progestational agents. Serum HDL decline is associated with increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, net effect depends on balance achieved between doses of estrogen and progestogen and nature and absolute amount of progestogen used. Consider amount of both hormones in the choice of an OC.

The dosage regimen prescribed should contain the least amount of estrogen and progestogen compatible with a low failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of estrogen.

PERSISTENCE OF RISK OF VASCULAR DISEASE. Two studies have shown persistence of vascular disease risk for ever-users of OCs. In a LI.S. study, MI risk after OC discontinuation persists for at least 9 years in women 40-49 years who had used OCs for five or more years, increased risk was not demonstrated in other age groups. In a study in Great Britian, the risk of developing cerebrovascular disease persisted for at least 6 years after OCs stopped, although excess risk was very small. Both studies used OC formulations with 50 micrograms or higher

of estrogens.

2. Estimates of Mortality from Contraceptive Use—A study using data from several sources concluded that with the exception of OC users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The possibility of increased mortality risk with age for OC users is based on data from the 1970s—but reported in 1983. However, current practice involves use of lower estrogen dose formulations combined with careful restriction of OC use to women without the various risk factors listed in this labeling.

Changes in practice and new data suggesting that cardiovascular disease risk with OCs may be less than previously observed prompted the Fertility and Maternal Health Drugs Advisory Committee to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with OC use after age 40 in healthy nonsmokers (even with newer low-dose formulations), greater potential health risks are associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if effective, acceptable contraception is not available.

The Committee concluded that the benefits of OC use by healthy nonsmoking women over 40 may outweigh the possible risks. Older women, as all women who take OCs, should use the lowest possible effective dose formulation.

formulation.

3. Carcinoma of the Reproductive Organs — Numerous epidemiological studies have looked at the incidence of breast, endometrial, ovarian and cervical cancer in women using OCs. Overwhelming evidence suggests that OC use is not associated with an increase in risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. A few studies show a slightly increased relative risk of developing breast cancer, although the methodology of these studies, including differences in examination of users and nonusers, and in age at start of use, has been questioned. Some studies suggest that OC use is associated with an increased risk of cervical intraepithelial neoplasia in some populations of women. However, controversy continues about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between OC use and breast and cervical cancers, a cause and effect relationship has not been established.

relationship has not been established.

4. Hepatic Neoplasia—Benign hepatic adenomas are associated with OC use, although incidence is rare in the U.S. Indirect calculations estimate attributable risk to be in the range of 3.3 cases/100.000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

British studies have shown an increased risk of hepatocellular carcinoma in long-term (> 8 years) OC users; these cancers are extremely rare in the U.S. and attributable risk (excess incidence) of liver cancers in OC users approaches less than one per million users.

5. Ocular Lesions — There are clinical case reports of retinal thrombosis with OC use. Discontinue OCs if there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions; undertake appropriate diagnostic and therapeutic measures immediately.

lesions; undertake appropriate diagnostic and therapeutic measures immediately.

6. Oral-Contraceptive Use Before or During Farty Pregnancy—Extensive epidemiological studies revealed no increased risk of birth defects when OCs used prior to pregnancy. Studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. OC-induced withdrawal bleeding should not be used as a pregnancy test. Do not use OCs during pregnancy to treat threatened or habitual abortion. Rule out pregnancy if two consecutive periods missed before continuing OC use. If patient has not adhered to prescribed schedule, consider pregnancy at time of first missed period. Discontinue OC if pregnancy confirmed.

7. Gallbladder Disease—Earlier studies reported an increased lifetime relative risk of gallbladder surgery in users of OCs and estrogens; more recent studies show that the relative risk of developing gallbladder disease among OC users may be minimal, which may be related to use of formulations with lower hormonal estrogen and propestogen doses.

OC users may be minimal, which may be related to use of formulations with lower hormonal estrogen and progestogen doses.

8. Carbohydrate and Lipid Metabolic Effects — OCs cause glucose intolerance in a significant percentage of users. OCs with greater than 75 µg of estrogen cause hyperinsulinism: lower estrogen doses cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance reffect varies with different agents). Observe prediabetic and diabetic women carefully while taking OCs. In non-diabetic women, OCs have no apparent effect on fasting blood glucose.

A small proportion of women will have persistent hypertriglyceridemia while on OCs. Changes in serum triglycerides and lipoprotein levels have been reported in OC users (see Warnings).

9. Elevated Blood Pressure — Increase in blood pressure has been reported in women on OCs; increase is more likely in older OC users and with continued use. Data show that incidence of hypertension increases with increasing quantities of progestogens.

likely in older OC users and with continued use. Data show that incidence of hypertension increases with increasing quantities of progestogens.

Encourage women with history of hypertension or hypertension-related diseases, or renal disease to use another contraceptive method. Monitor hypertensive women electing to use OCs closely, discontinue OC if significant blood pressure elevation occurs. For most women, elevated blood pressure returns to normal after OC stopped. No difference in occurrence of hypertension among ever- and never-users exists.

10. Headache — Discontinue OC and evaluate cause at onset or exacerbation of migraine, or if new pattern of headache (i.e. recurrent, persistent, severe) develops.

11. Bleeding irregularities — Breakthrough bleeding and spotting sometimes occur especially during first 3 months of use. Type and dose of progestogen may be important. Consider non-hormonal causes and take adequate diagnostic measures to rule out malignancy or pregnancy in event of breakthrough bleeding, as with any abnormal vaginal bleeding. If pathology excluded, time or a formulation change may solve the problem. In the event of amenorrhea, rule out pregnancy. Some women encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

Precautions

Precautions

1. Physical Examination and Follow Up—A complete medical history and physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical exams should include special reference to blood pressure. breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests in case of undiagnosed, persistent or recurrent athormal vaginal bleeding, conduct appropriate diagnostic measures to rule out malignancy. Monitor women with strong family history of breast cancer or who have breast nodules with particular care 2. Lipid Disorders.—Follow women being treated for hyperlipidemias closely if they elect to use OCs. Some progestogens may elevate LDL levels and may render control of hyperlipidemias more difficult. (See Warnings) 3. Lipier Function—Discontinue OC I flaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. Fluid Petention—OCs may cause some degree of fluid retention, Prescribe with caution, and only with careful monitoring, in patients with conditions possibly apgravated by fluid retention. 5. Emoland Disorders—I significant depression occurs stop medication and use alternate contraceptive method in attempts to determine if symptom is drug related. Observe carefully those with history of depression and stop drug if depression recurs to serious degree. 6. Contact Lenses—Otntact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding and menstrail rregularities are associated with concomitant rifampin use. A similar association, though less marked, is suggested with barbiturates, phenylbutazone, phenyton sodium, and possibly with griseofulvin, and biod components may be affected by OCs. a. Increased prothombin and fact

Information for the Patient — See Patient Package Labeling.

Adverse Reactions — An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, arterial thromboembolism; pulmonary embolism; myocardial infarction; cerebral hemorrhage; cerebral thrombosis; hypertension; gallbladder disease; hepatic adenomas or benign lives thrombosis. liver tumors.

There is evidence of an association between the following conditions and OC use, although additional

There is evidence of an association between the following conditions and OC use, although additional confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis and averse reactions have been reported in patients on OCs and are believed to be drug-related nausea; vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea, temporary infertility after treatment discontinued; edema, melasma which may persist, breast changes tenderness, enlargement, secretion, change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice, migraine; rash (allerigic), mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in OC users and the association is neither confirmed nor refuted: congenital anomalies; premenstrual syndrome; cataracts, optic neuritis; changes in appetite; cystitis-like syndrome, headache; nervousness; dizziness; hirsuitism; loss of scalp hair; erythema multiforme; erythema modosum; hemorrhagic eruption; vaginitis; porphyria; impaired renal function; hemolytic uremic syndrome; Budd-Chiari syndrome acne; changes in libido; colitis; sickle-cell disease; cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes.

Overdosage — Serious ill effects have not been reported following acute ingestion of large doses of OCs by

valve prolapse; lugus-like syndromes.

Overdosage — Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits — The following noncontraceptive health benefits related to OC use are supported by epidemiological studies that largely utilized OC formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol. Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of inon-deficiency anemia; decreased incidence of dysmenorrhea. Effects related to inhibition of ovulation; decreased incidence of functional ovarian cysts; decreased incidence of ectopic pregnancies. Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease; decreased incidence of endometrial cancer; decreased incidence of acute pelvic inflammatory disease; decreased incidence of endometrial cancer; decreased incidence of acute pelvic inflammatory disease; decreased incidence of endometrial cancer; decreased incidence of ovarian cancer.

Dosage and Administration — For maximum contraceptive effectiveness, take TRIPHASIL* (levonorgestrel and ethinyl estradiol tablets — triphasic regimen 21- and 28-day regimens) exactly as directed and at intervals not over 24 hours.

Indices 24 hours. If the property of the place on it until after the first menstrual cycle of medication or postpartum, contra-ceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.) For full details on dosage and administration see prescribing information in package insert.



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TRANSACTIONS OF THE ELEVENTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

The role of repeat glucose tolerance tests in the diagnosis of gestational diabetes

Ran Neiger, MD, and Donald R. Coustan, MD

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The diagnosis of gestational diabetes requires that two of the four 100 gm, 3-hour oral glucose tolerance test values be elevated. Our report evaluates the usefulness of repeating the oral glucose tolerance test in patients who have only one abnormal value. One hundred six patients who had abnormal results of diabetes screening tests (glucose level ≥ 130 mg/dl) and whose glucose tolerance test had one abnormal value underwent repeat glucose tolerance testing at an average of 4.6 weeks later. Thirty-six patients (34%) had two abnormal values on the repeat test and were classified as having gestational diabetes. Our results indicate that the finding of one abnormal value on a glucose tolerance test denotes a significant risk for the development of gestational diabetes. (AM J OBSTET GYNECOL 1991;165:787-90.)

Key words: Gestational diabetes, oral glucose tolerance test, plasma glucose levels

Gestational diabetes mellitus is defined as carbohydrate intolerance of variable severity with onset or first recognition during a pregnancy.1 Diagnosis and treatment of gestational diabetes may reduce perinatal mortality and morbidity.2 About half of patients whose oral glucose tolerance test (GTT) results are abnormal lack the risk factors traditionally considered to be markers for gestational diabetes.3 Therefore all pregnant women are now screened for gestational diabetes in many centers. Those whose screening tests results are abnormal are evaluated with a 3-hour, 100 gm oral GTT. The diagnosis of gestational diabetes requires at least two abnormal values on the oral GTT.

In an attempt to identify those patients whose initial oral GTT results are not diagnostic of gestational diabetes but in whom this disease may develop in a later initial oral GTT. Our hypothesis was that during the period between the two tests the glucose tolerance of some of these patients would worsen such that they would meet the criteria for gestational diabetes. Material and methods

stage of pregnancy, we investigated the results of repeat

oral GTTs in patients with one abnormal value on the

We screen all pregnant women for gestational diabetes at 24 to 28 weeks' gestation with a 1-hour, 50 gm glucose load. Our study population included women who had no history of diabetes and who had abnormal diabetes screening test results (glucose ≥130 mg/dl, plasma, hexokinase)4 and one abnormal value on the 3-hour, 100 gm oral GTT (defined as glucose values: fasting, ≥95 mg/dl; 1 hour, ≥180 mg/dl; 2 hours, ≥155 mg/dl; 3 hours, ≥140 mg/dl, plasma, hexokinase).5 We evaluated the likelihood of a diagnosis of gestational diabetes when the oral GTT was repeated approximately 1 month later. To evaluate the possibility of bias in the selection of patients for repeat testing, we also gathered data on subjects who, during the same period, had abnormal diabetes screening test results and one elevated oral GTT value but for various reasons did not have a follow-up GTT. Estimated gesta-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Brown University and Women and Infants'

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Table I. Demographic data

	Patients with "normal" repeat GTT results	Patients with abnormal repeat GTT results	p Value
No.	70	36	
Age (yr)	27.5 ± 5.1	27.7 ± 5.4	0.8
Gravidity	2.5 ± 1.9	2.3 ± 1.7	0.5
Parity	0.9 ± 1.3	0.7 ± 1.1	0.6
Mean prepregnancy weight (% ideal body weight)	104.4 ± 21.6	110.3 ± 24.8	0.2

Table II. Results of diabetes tests

	Patients with "normal" repeat GTT results	Patients with abnormal repeat GTT results	p Value
No.	70 -	36	
Gestational age at screening test (wk)	27.7 ± 1.7	26.9 ± 2.2	0.05
Screening test result (mg/dl)	149 ± 14	148 ± 14	0.9
Gestational age at first GTT (wk)	30.6 ± 1.9	29.8 ± 3.4	0.1
Delta* (mg/dl), WIH criteria	9 ± 8	12 ± 12	0.1
Delta (mg/dl), ACOG criteria	0 ± 9	2 ± 11	0.2
Gestational age at second GTT (wk)	35 ± 2	34.7 ± 2.2	0.5
Time interval (wk)	4.5 ± 1.1	5 ± 1.9	. 0.08

WIH, Women and Infants' Hospital; ACOG, American College of Obstetricians and Gynecologists.

tional ages at the time of diabetes testing and at delivery were based on the time elapsed since the patients' last menstrual periods, unless contradicted by ultrasonographic studies. Perinatal outcome variables also were recorded. Calculations of average birth weight included only singleton infants delivered at ≥ 37 weeks' gestation. Statistical analysis was done with Student's t test for independent means, and the level of significance was considered to be $p \leq 0.05$.

Results

Our study population included 106 patients. Thirty-six of them (34%) had gestational diabetes on the repeat oral GTT. Demographic characteristics are listed in Table I. The results of and gestational age at the diabetes screening test and the time of the first and second oral GTTs are listed in Table II. To compare the degree of abnormality of the elevated first oral GTT values of these subjects, we calculated a derived value, delta, which is the increment of the abnormal plasma glucose value over its respective upper-normal limit, for both the criteria recommended by the American College of Obstetricians and Gynecologists⁶ and those used at our institution,⁵ measured in milligrams per deciliter.

The abnormality during the initial oral GTT was the fasting value in seven patients and the 1-, 2-, and 3-hour values in 14, 11, and 4 patients, respectively. Of the other 70 patients, 27 (39%) again had one abnormal value on the oral GTT (Fig. 1). The distribution of the

elevated glucose values on the first oral GTT of this group was as follows: fasting, 3 patients; 1-hour, 13 patients; 2-hour, 7 patients; 3-hour, 4 patients. In 16 of the 27 patients (59%) the abnormal glucose value occurred at the same time interval on the second oral GTT as during the initial study. The remaining 43 patients had normal values at all four time periods on repeat oral GTTs.

Three patients who had one abnormal glucose value on the initial oral GTT and again on the second one underwent a third oral GTT at 37 to 39 weeks' gestation. On the third test two patients had two abnormal values and were diagnosed as having gestational diabetes; the other again had one abnormal glucose value. Another patient, whose second oral GTT had four normal values, had a third oral GTT because of morbid obesity. The third test, done at 40 weeks' gestation, gave two abnormal glucose values.

We compared the patients who had repeat oral GTTs with a group of patients whose oral GTTs had one abnormal value but for various reasons did not have this test repeated (Fig. 1). Of these 117 patients, we were able to obtain complete records of 108 (92%). The reasons for not having the test repeated were as follows: The oral GTT result was interpreted as "normal" by the physician (64 patients); the patient refused to have the test repeated, did not keep the appointment, or did not finish the test (e.g., vomited) (10 patients); the patient was delivered before the test was repeated (11

^{*}Delta, Increment of the abnormal plasma glucose value over its respective upper-normal limit.

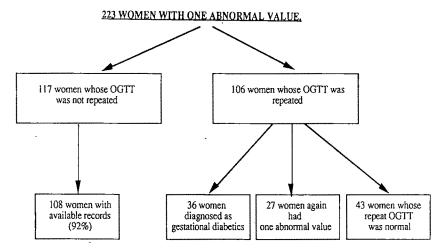


Fig. 1. Study population: 223 women with one abnormal oral GTT value.

patients); the patient was treated for gestational diabetes on the basis of one abnormal value (16 patients); reasons were unknown (7 patients). On comparison of the patients whose oral GTT was repeated with those who had only one oral GTT, there was no significant difference regarding gravidity (2.4 \pm 1.8 vs 2.4 \pm 1.5), parity (0.8 \pm 1.2 vs 0.9 \pm 1), timing and result of the diabetes screening test (27.5 \pm 1.9 vs 27.9 \pm 3.1 weeks and 148 ± 14 vs 151 ± 16 mg/dl, respectively; p = 0.2), and prepregnancy weight (106% ± 23% vs 105% ± 23% of ideal body weight). The degree of abnormality of the one elevated glucose value on the initial oral GTT also was similar (10 \pm 10 vs 12 \pm 14 mg/dl by our criteria⁵). Patients whose initial oral GTTs were repeated were younger than those who had this test only once $(27.6 \pm 5.1 \text{ vs } 29.1 \pm 5.2 \text{ years},$ p = 0.03). The first oral GTT was performed slightly but significantly later in pregnancy for those who had one oral GTT versus those whose tests were repeated $(31.9 \pm 3 \text{ vs } 30.3 \pm 2.5 \text{ weeks}, p < 0.001)$. Babies born to women whose oral GTTs were not repeated were not significantly heavier than those born to subjects who had repeat GTTs (3595 \pm 502 vs 3516 \pm 529 gm, p = 0.3).

Comment

The identification and treatment of gestational diabetes may reduce perinatal mortality and morbidity.7,8 The diagnosis of gestational diabetes is based on criteria originally proposed by O'Sullivan and Mahan,9 which include at least two abnormal glucose determinations on an oral glucose tolerance test. In our study we used the diagnostic criteria of O'Sullivan and Mahan converted for plasma glucose oxidase or hexokinase,5 which in a recent study by Sacks et al. 10 were found to be more consistent with the original criteria of O'Sullivan than were conversions proposed by the National Diabetes Data Group⁶ and endorsed by the American College of Obstetricians and Gynecologists.

The significance of a single abnormal glucose value on the oral GTT is not clear. Recent studies suggest that one abnormal value should be regarded as a pathologic finding. Langer et al.,11 using the National Diabetes Data Group criteria⁶ for normal oral GTT values, found that women with one abnormal value are at higher risk for adverse pregnancy outcomes. When untreated, such patients had a higher likelihood of large infants, as compared with patients whose oral GTT values were all normal. The infants born to patients with one abnormal value also had a significantly higher number of neonatal metabolic disorders. In another study¹² the incidence of preeclampsia or eclampsia was significantly greater in a group of patients with one abnormal oral GTT value, when compared with women whose screening tests results were normal. Tallarigo et al.13 found that even with normal oral GTT results, as defined by the National Diabetes Data Group criteria, higher 2-hour plasma glucose levels were associated with a significant increase in the incidence of macrosomia and congenital abnormalities, as well as toxemia, cesarean section, or both. Treating all patients who have one abnormal oral GTT value with a protocol of strict metabolic control was shown to be successful in reducing the incidence of large infants and metabolic problems to a level similar to that in normal control subjects.14

The data generated in this study support the hypothesis that the glucose tolerance of some patients who had one abnormal GTT value would worsen as pregnancy progressed. It is possible that patients whose gestational diabetes would have been diagnosed had the GTT been repeated make up a significant proportion of those who have large infants or whose infants have metabolic problems. If so, repeating the glucose tolerance test might have allowed for the avoidance of treatment in a significant number of patients, i.e., those whose GTT results remained normal, with the achievement of a similar good outcome for this group.

An alternative hypothesis is that the glucose tolerance test may not be a stable test with reproducible results and that patients in this study might even have had two abnormal values if the test had been repeated a day or two after its initial administration. Such a lack of stability has been demonstrated when this test was repeatedly performed on nondiabetic male volunteers. 15 A similar phenomenon was found when pregnant women were screened twice, on two successive days, with the 50 gm glucose test. 16 However, other studies demonstrated an increasing incidence of abnormal screening test results with advancing gestational age, when the same subjects were retested. 17, 18 These findings support the hypothesis that worsening glucose tolerance during the interval between the two GTTs caused the development of gestational diabetes in 34% of our patients.

Our results indicate that the presence of one abnormal value on the initial glucose tolerance test denotes a significant risk for the development of gestational diabetes. We recommend repeating the oral GTT 3 to 4 weeks after the initial study in such patients. Gestational diabetes may develop in a third of these women in the month after the first test. It is possible that the interval between tests is less important than the mere repetition of the oral GTT, a question that is unanswered in our study.

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Atrial natriuretic peptide: A vasodilator of the fetoplacental circulation?

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Paired maternal and fetal atrial natriuretic peptide concentrations were measured in 62 percutaneous umbilical blood samplings performed principally for the assessment and treatment of rhesus isoimmunization. Pretransfusion fetal atrial natriuretic peptide levels were significantly higher than maternal atrial natriuretic peptide levels (median 117 pg/ml vs median 32 pg/ml; p < 0.001); paired pretransfusion fetal and maternal atrial natriuretic peptide samples showed a weak correlation with each other ($R^2 = 17\%$; p = 0.002). Fetal atrial natriuretic peptide levels correlated inversely with hematocrit ($R^2 = 14\%$; p = 0.003), but not with albumin or gestational age. Paired pretransfusion and posttransfusion (median = 134 pg/ml) fetal atrial natriuretic peptide levels (n = 38) showed a significant rise after transfusion (p < 0.001); this rise was related to the percentage of fetoplacental blood volume transfused ($R^2 = 33\%$; p = 0.035). In a subgroup of 26 procedures, change in fetal atrial natriuretic peptide levels was weakly correlated with transient reductions in the Doppler systolic/diastolic ratio of the umbilical artery ($R^2 = 14\%$; p = 0.07). These data support work in animals that indicate a role for atrial natriuretic peptide in the human fetus, but these data do not confirm that atrial natriuretic peptide modulates fetoplacental vascular impedance in the human fetus. (Am J Obster Gynecol. 1991;165:791-800.)

Key words: Atrial natriuretic peptide, paired maternal and fetal samples, intravascular transfusion, Doppler ultrasonography systolic/diastolic ratio, umbilical artery, middle cerebral artery, aorta

The technique of intravascular transfusion therapy for rhesus isoimmunization has transformed the prognosis for this condition, especially when the fetus has hydrops.¹ During this procedure it is possible to virtually double the estimated fetoplacental blood volume in minutes; in an adult, especially an anemic one, this process would prove disastrous. The fetus is different because additional vascular capacity is provided by the unique fetoplacental circulation, which may respond to vasoactive substances and hence may protect the fetal cardiovascular system from overload.

A putative signal between fetus and placenta in this context may be atrial natriuretic peptide, the vasodilator, natriuretic, and diuretic properties of which have been well characterized.² Studies demonstrate atrial natriuretic peptide levels in fetal blood,³⁻⁶ which increase

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after intravascular transfusion, possibly in response to atrial stretch⁷; similar observations have been made with a sheep model.⁸

Specific binding sites for atrial natriuretic peptide have been identified within the placenta, 9-11 and physiologic atrial natriuretic peptide levels have been shown to reduce the vasoconstrictive effect of angiotensin II, 12 suggesting that fetal atrial natriuretic peptide (presumably released from the fetal heart) may have an endocrine action within the fetoplacental vasculature.

The circulatory adaptive events after transfusion of the fetus have been studied by several investigators using Doppler ultrasonography on the basis that the peak systolic—end-diastolic flow velocity ratio may reflect downstream vascular impedance.¹³ Umbilical arterial systolic/diastolic ratios have been shown to fall I hour after transfusion,¹⁴ and serial measurements of the pulsatility index from the umbilical artery and fetal vessels have demonstrated transient changes consistent with a reduction in downstream impedance.^{15, 16} Others, however, have not found such changes.¹⁷

The purpose of this study was to extend our original observations⁴ on the physiology of fetal atrial natriuretic peptide, in particular to examine the possible relationship between changes in fetal atrial natriuretic peptide levels and alterations in fetoplacental Doppler

ultrasonographic waveform indexes after intravascular transfusion.

Material and methods

Patient population. Thirty pregnancies were studied through 62 procedures. Of these, 27 women underwent percutaneous umbilical blood sampling for the assessment and treatment of red blood cell isoimmunization. Fourteen of the pregnancies provided serial data because of repeated transfusions. Doppler ultrasonographic studies were performed in association with 26 transfusions given to 10 of these patients. All fetuses were structurally and karyotypically normal.

Three additional women who underwent percutaneous umbilical blood sampling for fetal karyotype determination were included because their fetuses were structurally normal and had normal results of chromosomal analyses.

Collection of fetal blood samples. The ethics committee approved the study. Small samples of fetal blood (1 ml each) were initially routinely withdrawn for hematocrit, total protein, albumin, and karyotype determination with a 20-gauge spinal needle and needle guide under ultrasonographic guidance. All blood samples were taken from the umbilical vein, usually at the cord root. The purity of the fetal blood sample was checked against a maternal sample by determination of mean red blood cell volume (Chanelyzer, Coulter Electronics, Harpendon, Hertfordshire, England). An additional 1 ml of blood was taken at this point for radioimmunoassay of atrial natriuretic peptide; 1 ml of the maternal sample was used for comparison. Vecuronium bromide (0.2 to 0.4 mg) was used to paralyze the fetus before transfusion in 8 of 26 transfusions in the Doppler ultrasonography study group.

The volume of blood to be transfused was calculated on the basis of hematocrit, gestational age, and donor blood hematocrit, ^{18, 19} and included the volume to be withdrawn as samples (usually 3 to 5 ml). An additional sample to measure atrial natriuretic peptide levels was taken at the time of routine determination of posttransfusion hematocrit. A sample of donor blood was collected for measurement of atrial natriuretic peptide levels in 20 cases. Small (0.1 ml) capillary samples of donor and fetal (pretransfusion and posttransfusion) blood were obtained in 12 cases to determine pH and Po₂.

Radioimmunoassay for atrial natriuretic peptide. The samples were collected into tubes containing ethylenediaminetetraacetic acid and aprotinin on ice, separated by using a chilled centrifuge within 30 minutes, and stored at -20° C before analysis. We used the method we previously reported.²⁰ The plasma samples were initially preextracted; recovery of atrial natriuretic peptide was checked by the addition of iodine 125–

labeled atrial natriuretic peptide to control plasma and found to be >85% in each batch. The dried extracts were reconstituted in buffer, and atrial natriuretic peptide was determined by using a rabbit anti-atrial natriuretic peptide antibody. The interbatch coefficient of variation was <10%.

Measurement of fetoplacental Doppler waveforms. Measurements of the systolic-diastolic flow velocity ratios from the umbilical artery, aorta, and middle cerebral artery were obtained in a subgroup of 26 transfusions in 10 cases. Measurements were taken before transfusion (t = 0), and within 15 minutes (t = 1), at 6 hours (t = 2), and at 24 hours (t = 3) after transfusion. All measurements were taken by a single observer (G.R.) with a 3.5 MHz curvilinear probe with integralpulsed Doppler ultrasonography, color mapping, and M-mode capabilities (Spectra, Diasonics-Sonotron, Bedford, England). The high pass filter was set at 50 Hz. The technique of Mari et al.21 was used to locate the optimal signal from the middle cerebral artery, and we attempted to obtain an angle of insonation of <55 degrees for all aortic readings. In the absence of fetal breathing or movement, six consecutive waveforms were used to calculate the mean systolic/diastolic ratio at each site and the fetal heart rate.

Statistical analysis. Maternal and fetal pretransfusion and posttransfusion atrial natriuretic peptide levels were skewed, as is illustrated by the median, interquartile range, and limits of distribution (Fig. 1). A log (e) transformation allowed the atrial natriuretic peptide data to be adequately approximated by a normal distribution. Paired Student's *t* tests were used to compare differences between maternal and fetal atrial natriuretic peptide levels and fetal atrial natriuretic peptide levels before and after transfusion.

The factors influencing pretransfusion fetal atrial natriuretic peptide levels were determined by multiple regression analysis. Those variables considered to influence the change in fetal atrial natriuretic peptide levels after transfusion were analyzed in a similar manner. Relationships between other continuous variables were examined by simple linear regression and correlation. The results of these analyses are presented in terms of both R^2 and p values.

A summary (quadratic) measures analysis²² was used to analyze serial data (either heart rate or Doppler ultrasonographic systolic/diastolic ratio measurements) from each patient. In contrast to the frequently used method of comparing grouped data between individual time points, the summary measures method treats the subject (in this case each transfused fetus) as the basic unit, deriving a single number that summarized its response, the significance of which is examined with an unpaired Student t test. This method has distinct advantages in comparison with analysis of grouped data.²²

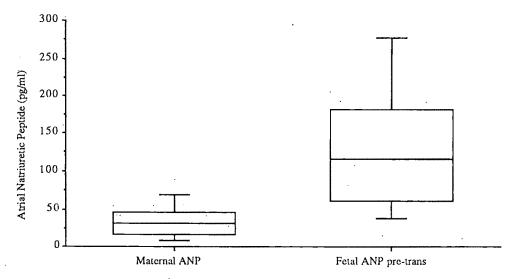


Fig. 1. Box plot of median, interquartile range, and limits of distribution of maternal and fetal pretransfusion atrial natriuretic peptide (ANP).

Our study tested the hypothesis that fetal atrial natriuretic peptide levels would rise so rapidly in response to intravascular transfusion that any effect of atrial natriuretic peptide on Doppler ultrasonographic waveforms would likely be maximal by 15 minutes and, from data with the sheep model,8 fetal atrial natriuretic peptide levels (and by implication their effects within the fetoplacental circulation) would have returned to basal levels ≤ 6 hours. An increase in circulating fetal atrial natriuretic peptide levels would be expected to facilitate blood flow; this should be represented by a reduction in observed Doppler ultrasonographic systolic/diastolic ratio. Thus a summary measure of initial reduction, followed by recovery in waveform systolic/diastolic ratio between t = 0 and t = 2 would describe the changes in systolic/diastolic ratios over time that might relate to the time course of atrial natriuretic peptide in the fetoplacental circulation. This is illustrated in Fig. 2. The significance of each derived summary measure (for heart rate and each of the three vessels studied) was examined by unpaired Student's t test.

Results

Maternal and fetal pretransfusion atrial natriuretic peptide. The plasma atrial natriuretic peptide levels results for paired maternal and fetal pretransfusion samples (n = 62) are shown in Fig. 1. Fetal atrial natriuretic peptide pretransfusion levels were significantly greater than (p < 0.001) and weakly correlated with paired maternal atrial natriuretic peptide levels (Fig. 3): $R^2 = 17\%$; p = 0.002).

Gestational age, total protein, albumin, and hematocrit were examined by multiple regression analysis to determine their independent influences on fetal atrial natriuretic peptide pretransfusion levels. Hematocrit

the most dominant variable $(R^2 = 14\%)$; p = 0.003), as is shown in Fig. 4. There was no evidence of a relationship between fetal atrial natriuretic peptide pretransfusion levels and either gestational age or total protein, whereas the relationship with fetal albumin was close to significant (p = 0.07). Among the 26 transfusions in the Doppler ultrasonography group, there was no relationship between pretransfusion atrial natriuretic peptide levels and pretransfusion heart rate (p = 0.82).

Change in fetal atrial natriuretic peptide after transfusion. The paired pretransfusion and posttransfusion fetal atrial natriuretic peptide levels are shown individually in Fig. 5 (n = 38). Fetal atrial natriuretic peptide levels increased after transfusion in most instances (median posttransfusion atrial natriuretic peptide level = 134 pg/ml), and this increase was highly significant (paired Student's t test p < 0.001). Atrial natriuretic peptide was undetectable (<3 pg/ml) in donor blood (n = 20).

Several factors thought to potentially influence the change in atrial natriuretic peptide levels after transfusion (log posttransfusion atrial natriuretic peptidelog pretransfusion atrial natriuretic peptide), such as volume of blood transfused, change in hematocrit, percentage of fetoplacental blood volume transfused, and pretransfusion atrial natriuretic peptide, were examined by multiple regression analysis, which demonstrated significant and independent relationships between change in fetal atrial natriuretic peptide levels and (I) the percentage of fetoplacental blood volume transfused ($R^2 = 33\%$; p = 0.035) and (2) pretransfusion fetal atrial natriuretic peptide levels ($R^2 = 13\%$; p = 0.01).

Donor blood was significantly acidotic (mean pH

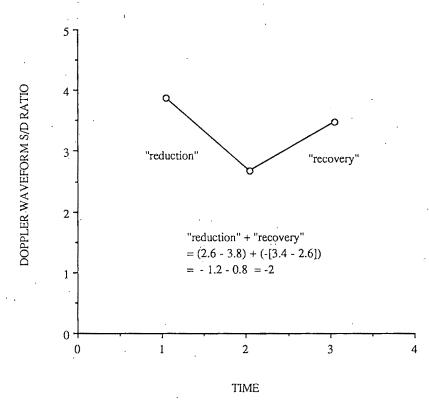


Fig. 2. Calculation of summary measure of change in waveform systolic/diastolic (S/D) ratio between three time points. Calculation is chosen to give a negative value for a response characterized by an initial reduction that is followed by a recovery.

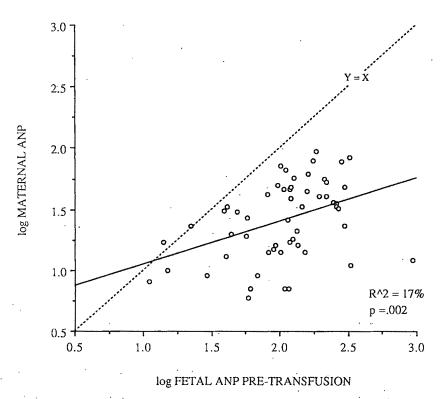


Fig. 3. Relationship between maternal and fetal pretransfusion atrial natriuretic peptide values. Note all but two lie below Y = X line, indicating that fetal atrial natriuretic peptide (ANP) levels are higher than paired maternal levels. *Continuous line* is simple regression between variables.

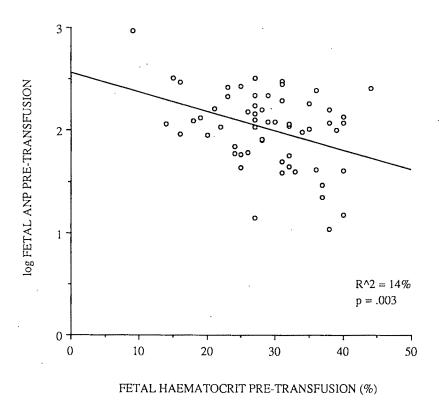


Fig. 4. Relationship between pretransfusion fetal atrial natriuretic peptide (ANP) levels and pretransfusion fetal hematocrit (p = 0.003).

6.91) compared with fetal blood pretransfusion (mean pH = 7.38; p < 0.001). In spite of transfusion (median percentage of fetoplacental blood volume given = 40%, n = 12) there was no significant change in fetal pH after this procedure (mean pH after transfusion 7.37). Similarly, the results for Po₂ showed no significant change after transfusion.

Changes in fetal heart rate and Doppler ultrasonographic waveform systolic/diastolic measurements after transfusion. The change in heart rate across each of the four time points is shown in Fig. 6, which shows an initial fall (between t = 0 and t = 1) followed by an increase (between t = 1 and t = 2). A summary measure analysis (Fig. 2) calculated for each fetus between time points 0 and 2 indicated a significant reduction and recovery in fetal heart rate after transfusion (mean change = -11.8 beats/min; unpaired Student's t test p < 0.001). This change in fetal heart rate was not associated with change in fetal atrial natriuretic peptide level ($R^2 < 1\%$; p = 0.82).

Because of the known influence of heart rate on the umbilical artery systolic/diastolic ratio (a reduction in heart rate will lower the systolic/diastolic ratio), all umbilical artery systolic/diastolic ratios were thus transformed to a standard heart rate of 140 beats/min.23 The corrected umbilical artery measurements for each fetus are shown in Fig. 7. The responses of those fetuses in whom an decrease in atrial natriuretic peptide level was observed after transfusion (n = 6) are indicated separately from the main group.

A summary measure of the corrected umbilical artery systolic/diastolic ratio and the systolic/diastolic ratios from both the middle cerebral artery and aorta for each fetus between time points 0 and 2 were derived (Fig. 2). The grouped summary measures data for each vessel studied are illustrated in Fig. 8; both the middle cerebral artery and aorta deviated significantly from baseline levels (unpaired Student's t test; middle cerebral artery, p = 0.002; aorta, p = 0.04), in contrast to the umbilical artery (p = 0.29).

The summary measure of the Doppler ultrasonographic waveform change from each vessel studied was compared with the change in fetal atrial natriuretic peptide levels (log after transfusion atrial natriuretic peptide level - log before transfusion atrial natriuretic peptide level) for each fetus. A simple regression between summary measure of the corrected umbilical artery systolic/diastolic ratio and change in fetal atrial natriuretic peptide levels (Fig. 9) is just above the conventional level of significance ($R^2 = 14\%$; p = 0.07). A similar analysis between either middle cerebral artery or aorta and change in fetal atrial natriuretic peptide levels demonstrated even weaker associations (middle cerebral artery: $R^2 = 8\%$, p = 0.21; aorta: $R^2 = 9\%$, p = 0.10), in spite of the more obvious change in waveforms in response to transfusion.

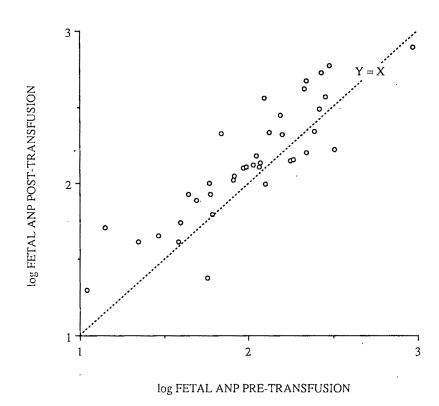


Fig. 5. Scatterplot of paired pretransfusion and posttransfusion fetal atrial natriuretic peptide (ANP) levels on a log (e) scale. Majority of data points are above the Y = X line, indicating higher posttransfusion values (p < 0.001).

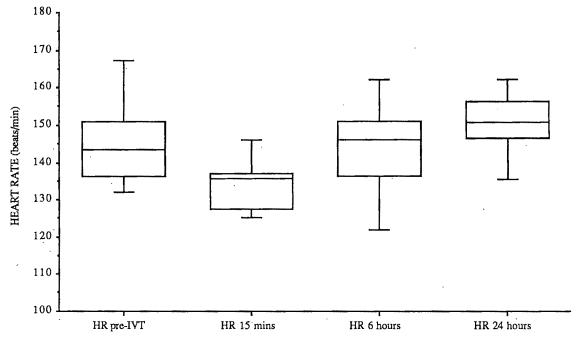


Fig. 6. Effect of intravascular transfusion (IVT) on fetal heart rate (HR) shown as grouped data. Summary measures analysis demonstrated significant changes between time points 0 and 2 (p < 0.001).

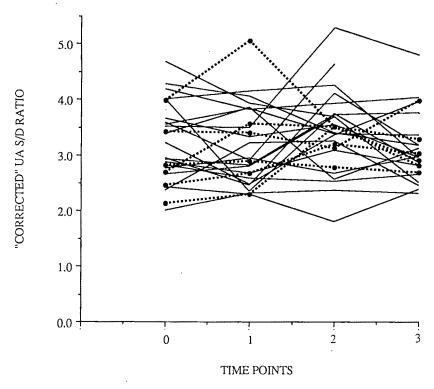


Fig. 7. Effect of intravascular transfusion on corrected umbilical artery (U/A) systolic/diastolic (S/D) ratios. -- • --, Cases in which decrease in fetal atrial natriuretic peptide levels was demonstrated after transfusion.

Comment

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Our study provides additional information concerning the existence of atrial natriuretic peptide in the fetoplacental circulation and attempts to relate biophysical changes (as observed with Doppler ultrasonography) to an endocrine response, namely change in circulating fetal atrial natriuretic peptide levels.

Fetal atrial natriuretic peptide levels were significantly higher than paired maternal levels, suggesting independent production by the fetoplacental circulation. Furthermore, the correlation we have shown between fetal and maternal atrial natriuretic peptide levels suggests either that atrial natriuretic peptide transfers across the placenta or that the placenta itself is a source of atrial natriuretic peptide for both fetus and mother. It seems unlikely that atrial natriuretic peptide transfers across the placenta because maternal atrial natriuretic peptide levels do not rise after intravascular transfusion.5 It may be possible that the placenta itself is a source of atrial natriuretic peptide for both fetus and mother, and increased placental production of atrial natriuretic peptide during pregnancy might explain the elevation of maternal atrial natriuretic peptide levels seen toward term.24

Fetal hematocrit (and to some extent fetal albumin)

has an inverse relationship with fetal atrial natriuretic peptide, suggesting that the release of atrial natriuretic peptide increases as fetal anemia progresses. The extreme of this relationship is found in hydrops when anemia is severe, and it is interesting that experimentally induced hydrops (by using the ovine model) is associated with substantial rises in fetal atrial natriuretic peptide levels.25 The release of atrial natriuretic peptide is normally governed by atrial stretch⁷; although the raised levels of fetal atrial natriuretic peptide found in hydrops may imply increased intravascular volume space, this has not been confirmed.18

The synthesis and release mechanisms in the human fetus are likely to be different from those in the adult. All four chambers of the fetal heart seem capable of atrial natriuretic peptide synthesis,26.27 and the possibility of extracardiac synthesis (for example, in the placenta) remains. The dominant mechanism controlling the release of fetal atrial natriuretic peptide is difficult to establish from our data, and it seems likely that several factors may be involved.

Intravascular transfusion caused a significant rise in fetal atrial natriuretic peptide levels in spite of the dilutional effect of the transfused donor blood, which contained negligible amounts of atrial natriuretic pep-

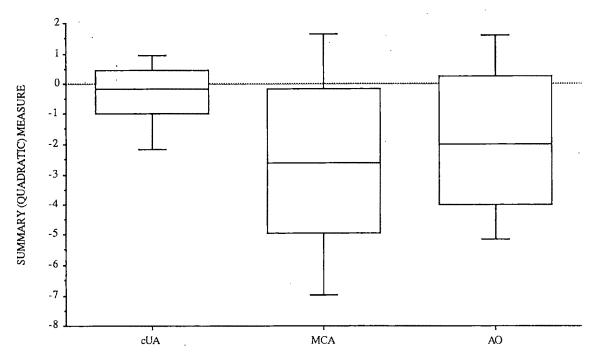


Fig. 8. Box plot of median, interquartile range, and limits of distribution for summary measures analysis of corrected umbilical artery (cUA), middle cerebral artery (MCA), and aorta (AO) waveform systolic/diastolic measurements between time points 0 and 2.

tide. It is interesting that the fetal atrial natriuretic peptide level of the baby with hydrops fell after transfusion. Fisk et al.⁶ made a similar observation, and we believe this occurs because the fetal heart has reached its maximal release rate of atrial natriuretic peptide in hydrops; thus the fall observed after transfusion is caused by dilution with donor blood.

In our study fetal atrial natriuretic peptide level responses to intravascular transfusion were significantly associated with volume transfused when expressed as a percentage change in estimated fetoplacental blood volume, suggesting that the release of atrial natriuretic peptide is related to mechanical stretch in the atria resulting from rapid intravascular transfusion along the umbilical vein. Panos et al.⁵ suggested an association between rate of transfusion and change in fetal atrial natriuretic peptide levels, although for technical reasons we believe this to be a difficult factor to measure accurately. Weiner et al.28 recently demonstrated that umbilical venous pressure rises transiently during intravascular transfusion. Umbilical venous pressure is likely to be closely related to the release of fetal atrial natriuretic peptide because it would more likely reflect change in right atrial pressure. As these authors suggest, the fall in venous pressure during transfusion might be related to vasodilatation within the placenta.

The most profound cardiovascular change in response to intravascular transfusion was a slowing of the fetal heart, which was previously described in ovine experiments²⁹ and which probably reflects a vagally mediated response to a rise in blood pressure. The change in fetal atrial natriuretic peptide level after transfusion was unrelated to this heart rate response, which is in broad agreement with recent data from a sheep model.³⁰

The relationship between change in fetal atrial natriuretic peptide levels and Doppler velocimetry of the fetal and umbilical vessels has been tested by using a summary (quadratic) measures analysis, which evaluates changes over time of the Doppler ultrasonography results. Because the fetal heart rate changes (which were not influenced by the use of vecuronium bromide) may have influenced the umbilical waveform systolic/diastolic ratios,²³ a correction was applied to a standard heart rate of 140 beats/min.

The changes in Doppler ultrasonography systolic/diastolic ratios from both the aorta and middle cerebral artery (Fig. 8) contrast those obtained from the umbilical artery. This observation may reflect a lack of innervation within the placenta, such that the fetoplacental circulation is protected from reflex-mediated changes in vascular impedance that likely operate within the fetus in response to intravascular transfusion. This phenomenon would allow relatively stable perfusion within the placenta, thereby protecting gas and nutrient transfer to the fetus.

In spite of these Doppler ultrasonographic waveform responses, there was only a weak and nonsignificant

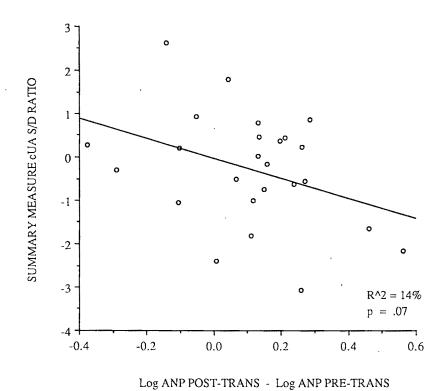


Fig. 9. Relationship between summary measure of change in corrected umbilical artery (cUA) systolic/diastolic (S/D) ratio (between time points 0 and 2) and change in fetal atrial natriuretic peptide (ANP) levels after transfusion (p = 0.07).

inverse relationship between change in atrial natriuretic peptide level and change in the umbilical artery waveform systolic/diastolic ratio. The lack of such an association is disappointing, but probably reflects the complex factors that may operate within the fetal cardiovascular system in response to intravascular transfusion. In support of this view are the observations by Brace et al.,30 who concluded from sheep experiments that the primary physiologic actions of atrial natriuretic peptide (vasodilatation and diuresis) may to a large extent be overshadowed by central cardiovascular changes. Thus it may not be surprising that we failed to demonstrate a strong relationship between elevations in fetal atrial natriuretic peptide level and implied changes in fetoplacental vascular impedance.

Another important aspect is that other vasoactive factors may operate to help the fetus cope with intravascular transfusion. Weiner and Robillard31 have shown an increase in the stable metabolite of the vasodilator prostanoid prostacyclin in fetal blood after intravascular transfusion. Moreover, this increase correlated with the rise in umbilical venous pressure, supporting the mechanism of shear-induced production of prostacyclin by the endothelial cell,32 presumably as a consequence of rapid transfusion into the umbilical vein.

The relative importance of vasoactive peptides and prostanoids (or other factors) in helping the fetus cope with intravascular transfusion remains an open question. It is hoped that future work will continue to relate biophysical data (Doppler ultrasonography or pressure measurements) to changes in putative endocrine or paracrine signals, so that we advance our understanding of fetoplacental circulation.

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Enhanced endothelium-derived relaxing factor activity in pregnant, spontaneously hypertensive rats

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Pregnancy is normally associated with vasodilation that, in hypertensive animals such as the spontaneously hypertensive rat, causes a profound decrease in blood pressure. To test the possibility that enhanced basal endothelium-derived relaxing factor activity has a role in the vasodilation of pregnancy, we measured the changes in mean arterial pressure and heart rate induced by N^g-monomethyl-L-arginine, a specific inhibitor of endothelium-derived relaxing factor synthesis, in conscious nonpregnant and pregnant (postmating day 20 to 21) normotensive Wistar-Kyoto and spontaneously hypertensive rats. No-monomethyl-L-arginine caused similar dose-dependent increases in mean arterial pressure in nonpregnant and pregnant Wistar-Kyoto rats, but the accompanying decrease in heart rate was significantly greater in nonpregnant rats than in pregnant ones. In the spontaneously hypertensive rats, Nª-monomethyl-L-arginine caused significantly greater dose-dependent increases in mean arterial pressure in pregnant compared with nonpregnant rats; there were no differences in the decreases in heart rate. These pressor responses were partially reversed by excess L-arginine but not D-arginine. Indomethacin had no effect on the pressor response to N^G-monomethyl-L-arginine or the depressor response to L-arginine after N^a-monomethyl-L-arginine. Therefore basal endothelium-derived relaxing factor plays a role in vascular tone and blood pressure regulation in vivo, and pregnancy may be associated with enhanced basal endothelium-derived relaxing factor activity in the hypertensive spontaneously hypertensive rats. (Am J OBSTET GYNECOL 1991;165:801-7.)

Key words: Pregnancy, vasodilation, endothelium, blood pressure, hypertension, N^c-monomethyl-L-arginine

Pregnancy, in rodents and in humans, is associated with a decrease in systemic vascular resistance. ^{1,2} Consequently, maternal blood pressure remains constant, or is moderately decreased, even though blood volume and cardiac output increase 40% to 50%. The factor(s) responsible for pregnancy vasodilation is still not known with certainty and remains the subject of intense investigation.

The vascular endothelium plays a prominent but complex role in the regulation of vascular tone and reactivity through production of a number of paracrine vasoactive substances. Since the demonstration in 1980 that vascular relaxation induced by acetylcholine was dependent on the presence of a functionally intact endothelium, numerous other agonists (e.g., bradykinin, adenosine diphosphate, histamine, and thrombin) and physical stimuli (e.g., fluid shear force and pulsatile

flow) have been shown to stimulate endothelial cell production and release of a labile endothelium-derived relaxing factor.3 Endothelium-derived relaxing factor is believed to be nitric oxide, or a nitric oxide-containing S-nitrosothiol, derived from the oxidation of the guanidino nitrogen atom of the amino acid L-arginine.5 It diffuses readily into adjacent vascular smooth muscle cells and stimulates soluble guanylate cyclase production of cyclic 3'5'-guanine monophosphate with resultant relaxation.6 Endothelium-derived relaxing factor also inhibits platelet aggregation by stimulating platelet cyclic 3'5'-guanine monophosphate production.7 Analogs of L-arginine, such as No-monomethyl-L-arginine, competitively inhibit synthesis of endothelium-derived relaxing factor and block endothelium-dependent relaxation both in vitro5. 8. 9 and in vivo. 10. 11 Furthermore, administration of NG-monomethyl-L-arginine intravenously causes a sustained increase in blood pressure with moderate bradycardia, strongly suggesting that basal endothelium-derived relaxing factor production plays a role in the regulation of vascular tone in vivo. 10-12

Recent observations that the endothelium-dependent vasodilatory response to acetylcholine was enhanced in arteries from pregnant compared with non-pregnant animals^{13, 14} have suggested the possibility that an augmentation of basal or stimulated endothelium-

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 99.8 ± 7.6

 185.1 ± 3.2

 $138.7 \pm 2.3*$

113.3 ± 5.6*

206.5 ± 3.8*

 $168.3 \pm 5.7*$

 91.3 ± 4.4

 178.3 ± 4.8

(p < 0.001)

 118.1 ± 4.0

Table I. Effects of N^G-monomethyl-L-arginine, D-arginine, and L-arginine on mean arterial blood pressure and heart rate in nonpregnant and term-pregnant normotensive Wistar-Kyoto and spontaneously hypertensive rats

Pregnant (n = 6)

Nonpregnant (n = 8)

Spontaneously hypertensive rats

derived relaxing factor activity may be responsible for the vasodilation of pregnancy. Furthermore, plasma levels and urinary excretion of cyclic 3'5'-guanine monophosphate are increased early in pregnancy in rats, suggesting that vascular production may be enhanced. ¹⁵ However, intravenous administration of N^c-monomethyl-L-arginine produced similar pressorresponses in term-pregnant and nonpregnant rats, suggesting that basal endothelium-derived relaxing factor production is not enhanced during pregnancy. ¹⁶

To further test the hypothesis that basal endothelium-derived relaxing factor activity is enhanced during pregnancy, we measured the pressor responses to N^Gmonomethyl-L-arginine in nonpregnant and termpregnant spontaneously hypertensive rats. These rats, a genetic model of human essential hypertension, retain the ability to vasodilate normally during pregnancy, and blood pressure falls to normotensive levels by the day before parturition.¹⁷ For comparison, we also measured the pressor responses to NG-monomethyl-Larginine in nonpregnant and term-pregnant normotensive Wistar-Kyoto rats. To test the possibility that prostaglandins may contribute to these responses, we also measured the pressor responses to N⁶monomethyl-L-arginine in indomethacin-treated nonpregnant and term-pregnant spontaneously hypertensive and Wistar-Kyoto rats.

Material and methods

Virgin female spontaneously hypertensive and Wistar-Kyoto rats (10 to 12 weeks old) were purchased from Harlan Laboratories, Inc. (Indianapolis, Ind.). The endothelium-derived relaxing factor inhibitor Nomonomethyl-L-arginine acetate was obtained from Peninsula Laboratories, Inc. (Belmont, Calif.). L-Arginine and D-arginine were obtained from Sigma Chemical Co. (St. Louis). Indomethacin trihydrate was provided by Merck, Sharp and Dohme (Rahway, N.J.). This project conforms with the "Guiding Principles in the Care

and Use of Laboratory Animals" approved by the Council of the American Physiological Society and with federal laws and regulations. The protocol used was approved by the University of Tennessee Animal Care and Use Committee.

112.5 ± 5.4*

 $202.5 \pm 2.9*$

165.9 ± 4.2*

The animals were housed in a room with temperature maintained at $22^{\circ} \pm 1^{\circ}$ C and with a 12-hour-light/12-hour-dark cycle (lights on from 6 AM to 6 PM). They were fed standard laboratory rodent chow (Ralston Purina, St. Louis) as desired with tap water to drink. Females were caged 1:1 with mature males of the same strain, and vaginal smears were checked daily in the early morning for the presence of spermatozoa. The day spermatozoa were found was designated postmating day 0. The timed-pregnant rats were subsequently housed one per cage and fed as desired until the day of experimentation (postmating day 20 or 21). Age-matched, unbred females of each strain were used for nonpregnant spontaneously hypertensive and Wistar-Kyoto rats.

Five days before experimentation, the rats were anesthetized with methoxyflurane and polyurethane catheters (Braintree Scientific, Braintree, Mass.) placed in the left carotid artery and the right external jugular vein. The catheters were tunneled subcutaneously to the back of the neck, exteriorized through a small incision between the scapulas, and fixed in place with suture and cyanoacrylate (Super Glue, B. Jadow and Sons, Inc., New York). The catheters were filled with heparinized (1000 U/ml) 0.9% saline solution and plugged with stainless steel pins. The rats were injected with penicillin G procaine and benzathine (30,000 IU per rat, intramuscularly) prophylactically.

On the day of experimentation, the rats were weighed and placed in an adjustable stainless steel holding cage designed to limit movement without causing undue stress, and the arterial catheter was connected to a pressure transducer (Statham P23Gb, Gould Electronics, Inc., Cleveland). Blood pressure was recorded

Pregnant (n = 7)*p < 0.001 versus baseline.

	Heart rate (bea	Heart rate (beats/min)				
Baseline	N ^G -monomethyl-L-arginine (40 mg/kg)	D-Arginine (100 mg/kg)	t-Arginine (100 mg/kg)			
460.0 ± 10.9 ($p < 0.05$)	356.7 ± 22.3*	341.7 ± 18.9*	373.3 ± 14.5*			
496.7 ± 9.5	441.7 ± 19.6*	440.0 ± 21.3*	$461.6 \pm 16.4*$			
492.5 ± 14.2 ($p < 0.01$)	$396.3 \pm 14.4*$	390.0 ± 13.0 *	433.8 ± 13.2*			
518.6 ± 6.4	$431.4 \pm 14.4*$	442.9 ± 12.7*	$475.7 \pm 13.1*$			

continuously with a Gould 2200S physiologic chart recorder (Gould). After blood pressure stabilization (about 1 hour), the experimental procedure was begun. In the first experiment baseline blood pressure was recorded for 5 minutes, followed by intravenous administration of cumulative doses of N^G-monomethyl-Larginine (5 to 40 mg/kg) at 5-minute intervals. Ten. minutes after the final dose, p-arginine (100 mg/kg) was administered intravenously, followed 10 minutes later by L-arginine (100 mg/kg). Mean arterial pressure was measured when blood pressure stabilized after each injection.

In the second experiment, to determine if prostaglandins play a role in the pressor response to Nomonomethyl-L-arginine, baseline blood pressure was recorded for 5 minutes after stabilization. Indomethacin (5 mg/kg) or 0.9% saline solution (untreated controls) was then administered intravenously. After I hour No-monomethyl-L-arginine (50 mg/kg) was administered intravenously, followed 30 minutes later by L-arginine (100 mg/kg). Mean arterial pressure was measured when blood pressure stabilized after each injection.

Mean arterial pressure was determined as diastolic pressure plus one third of the difference between systolic and diastolic pressure. Heart rate was read directly from the pulse pressure tracing. The maximum effect of N^G-monomethyl-L-arginine was estimated by the y intercept of the regression line of the double reciprocal plot of dose versus effect. The data are expressed as mean ± SEM. The data were analyzed by two-factor analysis of variance, and individual differences between means were determined by the least-squares means method. A value of $p \le 0.05$ was accepted as statistically significant for all analyses.

Results

Mean arterial pressure of the term-pregnant Wistar-Kyoto rats was moderately (≈35 mm Hg) but significantly lower than that of the nonpregnant Wistar-Kyoto rats (Table I). Mean arterial pressure of the term-pregnant spontaneously hypertensive rats was profoundly reduced (≈60 mm Hg) compared with that of the nonpregnant spontaneously hypertensive rats, however, and was not significantly higher than that of the nonpregnant normotensive Wistar-Kyoto rats. Pregnancy was associated with a slight, but significant, increase in heart rate in both strains of rats.

No-Monomethyl-L-arginine induced a dose-dependent increase in mean arterial pressure accompanied by a decrease in heart rate in both Wistar-Kyoto and spontaneously hypertensive rats. In the Wistar-Kyoto rats, it induced similar increases in blood pressure in both nonpregnant and pregnant rats (Fig. 1). There was no significant difference in estimated maximum effect. After the last dose of No-monomethyl-L-arginine (cumulative dose of 40 mg/kg), the decrease in heart rate was significantly greater in the nonpregnant rats compared with the pregnant Wistar-Kyoto rats (Table I, Fig. 1). In the spontaneously hypertensive rats N^cmonomethyl-L-arginine induced significantly greater increases in mean arterial pressure in the pregnant rats than in the nonpregnant rats at all doses (Fig. 2), and after the final dose the mean arterial pressure of the pregnant spontaneously hypertensive rats was not significantly lower than that of the nonpregnant spontaneously hypertensive rats before No-monomethyl-L-arginine (Table I). There were no differences between nonpregnant and pregnant spontaneously hypertensive rats in the No-monomethyl-L-arginine-induced decreases in heart rate (Fig. 2).

Excess L-arginine (100 mg/kg), but not D-arginine, partially restored mean arterial pressure to baseline levels in pregnant and nonpregnant spontaneously hypertensive and Wistar-Kyoto rats (Table I), confirming that the pressor responses to No-monomethyl-L-arginine were the result of inhibition of endothelium-derived relaxing factor synthesis from L-arginine. L-arginine had little effect on heart rate, however.

Indomethacin (5 mg/kg) pretreatment did not significantly change mean arterial pressure in either nonpregnant or pregnant Wistar-Kyoto or spontaneously

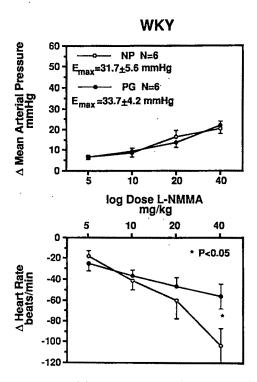


Fig. 1. Mean arterial pressure (top) and heart rate (bottom) effects of cumulative doses of N^G -monomethyl-L-arginine (L-NMMA) administered intravenously into nonpregnant and pregnant Wistar-Kyoto (WKY) rats. Data expressed as mean \pm SEM.

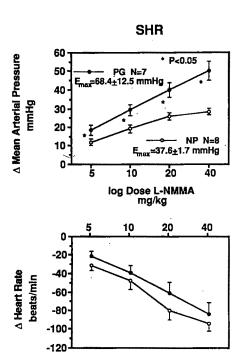


Fig. 2. Mean arterial pressure (top) and heart rate (bottom) effects of cumulative doses of N^G -monomethyl-L-arginine (L-NMMA) administered intravenously into nonpregnant and pregnant spontaneously hypertensive rats (SHR). Data expressed as mean \pm SEM.

hypertensive rats (Figs. 3 and 4). Furthermore, indomethacin pretreatment did not alter the pressor responses to N^G-monomethyl-L-arginine (50 mg/kg) or the depressor responses to L-arginine (100 mg/kg) in nonpregnant or pregnant spontaneously hypertensive or Wistar-Kyoto rats. Thus prostaglandins do not contribute to these responses.

Comment

These results confirm that rat pregnancy, like human gestation, is associated with systemic vasodilation and a decrease in maternal blood pressure. By postmating day 21, the day before expected delivery, mean arterial pressure was slightly lower and heart rate was slightly higher than in nonpregnant normotensive Wistar-Kyoto rats (Table I). They also confirm that, in the genetically hypertensive spontaneously hypertensive rat, a popular model of human essential hypertension, pregnancy has a profound antihypertensive effect. ¹⁷ By postmating day 21, blood pressure had reached normotensive levels in these rats.

The role of endothelial cell-derived autocrineparacrine substances such as prostacyclin and endothelium-derived relaxing factor in the regulation of vascular tone has been investigated extensively during the last two decades. Although vascular prostacyclin production is increased during pregnancy, there is no conclusive evidence that it is responsible for the decrease in systemic vascular resistance in the rat.18 Recent observations of enhanced endothelium-dependent relaxation responses to acetylcholine by arteries from pregnant rats¹³ and guinea pigs,¹⁴ and the observation of increased plasma levels and urinary excretion of cyclic 3'5'-guanine monophosphate, the second messenger of endothelium-derived relaxing factor-mediated vasodilation,15 has led to consideration that basal endothelium-derived relaxing factor production may be increased during pregnancy.

If basal endothelium-derived relaxing factor plays a role in the decreased vascular resistance during pregnancy, then we would expect No-monomethyl-L-arginine to cause a greater pressor response in pregnant rats compared with nonpregnant rats. In normotensive Wistar-Kyoto rats No-monomethyl-L-arginine induced similar moderate increases in mean arterial pressure in both nonpregnant and pregnant rats (Table I). Whereas these results support the hypothesis that basal endothelium-derived relaxing factor activity plays a role in vascular tone and blood pressure regulation in vivo, they do not support the hypothesis that increased endothelium-derived relaxing factor production plays a role in decreasing vascular tone during pregnancy. Our results are comparable to those of Umans et al.,16 using normotensive Sprague-Dawley rats. In their study an even higher dose of No-monomethyl-L-arginine (150 mg/kg) caused a 30 to 40 mm Hg increase

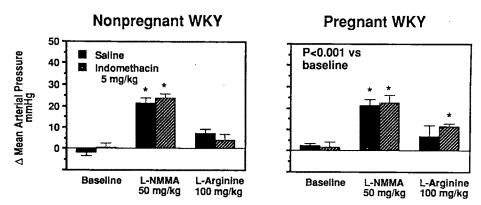


Fig. 3. Changes in mean arterial pressure (mean ± SEM) induced by N^G-monomethyl-L-arginine (L-NMMA) and L-arginine in untreated and indomethacin-treated, nonpregnant and pregnant Wistar-Kyoto (WKY) rats.

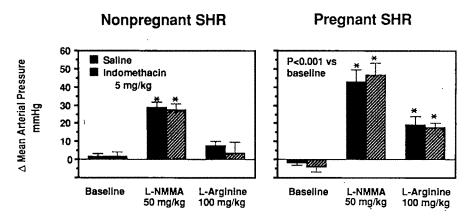


Fig. 4. Changes in mean arterial pressure (mean ± SEM) induced by N^G-monomethyl-L-arginine (L-NMMA) and L-arginine in untreated and indomethacin-treated, nonpregnant and pregnant spontaneously hypertensive rats (SHR).

in mean arterial pressure in both nonpregnant and pregnant rats. They concluded that pregnancy may not be associated with augmented basal endothelium-derived relaxing factor release and that basal and stimulated (e.g., by acetylcholine) endothelium-derived relaxing factor production may be regulated independently. Alternatively, the normotensive rat may already be in a generally vasodilated state with little need for further generalized vasodilation. The degree to which systemic vasodilation lowers blood pressure in the pregnant, normotensive rat is estimated to be very low, with decrements reported to range from 3 mm Hg19 to 17 mm Hg.20 However, in this study we observed a difference of approximately 35 mm Hg (Table I). Additional studies, including the effect of No-monomethyl-L-arginine on total periperhal resistance, are necessary to determine if basal or stimulated endothelium-derived relaxing factor plays a role in pregnancy vasodilation in normotensive rats.

The adult spontaneously hypertensive rat, on the other hand, is in a state of generalized vasoconstriction

with normal cardiac output. We have already demonstrated that pregnancy is associated with an increase in cardiac output and a decrease in the resistances of virtually all the vascular beds in the body,17 resulting in a large decrease in blood pressure (60 to 80 mm Hg) to normotensive levels by the day before delivery. Thus it was thought that this would be a good model in which to test the hypothesis that basal endothelium-derived relaxing factor activity is increased during pregnancy. Since all doses of No-monomethyl-L-arginine caused significantly greater increases in mean arterial pressure, with similar decreases in heart rate, in the pregnant compared with the nonpregnant spontaneously hypertensive rats (Table I, Fig. 2), it would appear that pregnancy may indeed augment basal endothelium-derived relaxing factor release in these rats. An alternative explanation for these results may be that vascular smooth muscle responsiveness to endothelium-derived relaxing factor is enhanced during pregnancy. This is considered unlikely, however, because preliminary results indicate that there is no difference between pregnant

and nonpregnant spontaneously hypertensive rats in the whole-body depressor response to sodium nitro-prusside (unpublished results). Sodium nitroprusside, like nitroglycerin, directly stimulates vascular smooth muscle-soluble guanylate cyclase and production of cyclic 3'5'-guanine monophosphate.²¹ Another alternative is that N^G-monomethyl-L-arginine may cause an increase in sympathetic nerve activity that may be of greater magnitude in pregnant than nonpregnant rats. Recent results in dogs have revealed a small but significant centrally mediated sympathoexcitatory effect of N^G-monomethyl-L-arginine administered intravenously.²² This possibility remains to be tested.

The fact that a molar excess of L-arginine, but not D-arginine, partially reversed the pressor responses to NG-monomethyl-L-arginine is further substantiation that the L-arginine/endothelium-derived relaxing factor pathway plays a role in vascular tone regulation in vivo. Since indomethacin did not cause any significant change in mean arterial pressure in either pregnant or nonpregnant Wistar-Kyoto or spontaneously hypertensive rats (Figs. 3 and 4), in agreement with Conrad and Colpoys, 18 we find no evidence that prostaglandins contribute to the vasodilation of pregnancy in these rats. Furthermore, since indomethacin pretreatment had no effect on the pressor response to NG-monomethyl-Larginine or the depressor response to L-arginine, neither vasoconstricting nor vasodilating prostaglandins play a role in these respective responses. Our results therefore suggest that endothelium-derived relaxing factor, not prostacyclin,23 is the antihypertensive factor of pregnancy in spontaneously hypertensive rats.

In summary, the results of this study confirm the results of others that basal endothelium-derived relaxing factor production plays a prominent role, perhaps more so than prostaglandins,24 in regulating vascular tone and blood pressure in vivo. They also support the hypothesis that pregnancy augments endothelium-derived relaxing factor activity in hypertensive spontaneously hypertensive rats. Furthermore, these results indicate that the functional integrity of the vascular endothelium, particularly with regard to the L-arginine-endothelium-derived relaxing factor pathway, may be particularly important in normal regulation of vascular tone during pregnancy in the hypertensive individual. Endothelial dysfunction and decreased production of endothelium-derived relaxing factor during pregnancy may play a role in the pathogenesis of preeclampsia.25

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Insignificant transfer of glyburide occurs across the human placenta

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No data exist concerning human placental transfer of oral hypoglycemic agents during pregnancy. This study characterizes the transport of glyburide in 10 term human placentas with the single-cotyledon placental model. Serial samples were taken from both the maternal and fetal reservoirs during each 3-hour perfusion, and the percent transport and metabolism of tritiated glyburide was calculated with liquid scintillation spectrometry and high-performance liquid chromatography. Antipyrine labeled with carbon 14 was added to the perfusate solution during these experiments as a control. Virtually no transfer of glyburide occurred, and no appreciable metabolism of the drug was detected. Neither variation in the albumin concentration nor increase in the maternal glyburide levels to 100 times therapeutic concentration materially altered the rate of transport. These data show that insignificant transport of glyburide occurs across the human placenta in vitro and suggest that fetal exposure to maternally administered glyburide likewise may be insignificant. (Am J Obstet Gynecol 1991;165:807-12.)

Key words: Glyburide; diazepam; pregnancy, human; placenta

Stringent glycemic control is the main therapeutic goal in the management of the pregnant patient with diabetes. Insulin remains the drug of choice in the 10% to 60% of diabetics with failure to maintain euglycemia with diet therapy alone. Furthermore, some authors recommend the prophylactic use of insulin in all women with gestational diabetes. Although it is believed

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that insulin itself does not cross the placenta, concern has recently been raised by the demonstration that insulin can readily cross the placenta as insulin-antibody complexes and possibly cause macrosomia.³

Although other modalities of management exist (e.g., oral hypoglycemic agents), their use in pregnancy is not well established. Furthermore, no study has addressed the issue of placental transfer of these drugs in humans. This has resulted in the exclusion of their use in the United States because of the fear of adverse pregnancy outcome.

Glyburide is one of the most commonly used secondgeneration sulfonylureas. Its molecular weight of 494 units of measure makes it one of the largest of the available oral hypoglycemics.⁴ Peak serum levels after a single oral dose of 5 mg occur at 4 hours and range from 112 to 360 ng/ml. Glyburide is extensively bound to albumin (98% to 99%), and metabolism occurs through hydroxylation in the liver to inactive metabolites.^{5, 6}

There is little information about the transplacental passage of oral hypoglycemic agents in humans. Disorders such as fetal anomalies, macrosomia; and neonatal hypoglycemia have been attributed to their use, 7.8 but these assumptions have not been confirmed by specific correlation with the degree of maternal-to-fetal transfer of these drugs. This study was undertaken to characterize the transport and metabolism of glyburide across the human placenta.

Methods and material

Tritiated glyburide was donated by The Upjohn Company, Kalamazoo, Mich. The purity of the substance was assured by high-performance liquid chromatographic fractionation, and identification of the peak fraction was done with the radioisotope. Stability of the compound in the perfusate solutions was tested by incubation of the compound in Krebs-Ringer buffer at pH 7.4 for 4 hours, and again the amount of parent compound was identified by joint high-performance liquid chromatography and radioimmunoassay.

Placental perfusions. The recirculating single cotyledon human placental model, as described by Brandes et al.,9 was used with minor modifications. Placentas were obtained from uncomplicated term gestations within 10 minutes of delivery. An appropriate cotyledon was chosen, and the supplying fetal artery and vein cannulated with 8 Fr. catheters. The cotyledon was then perfused with heparinized Krebs-Ringers buffer at pH 7.4. The circuit was checked for proper flow and absence of leaks. The placenta was then placed in the maternal chamber of the apparatus, and the maternal intervillous space was perfused by blunt cannulas at a flow rate of about 10 ml/min and a pressure of 1.5 cm Hg. The fetal circuit was perfused at a rate of approximately 2 ml/min and a pressure of 3.5 cm Hg. Albumin concentration in the maternal and fetal perfusates was varied between 0.2 and 2.0 gm/dl. Depending on the direction of transport being tested, the concentration of glyburide in either the maternal or fetal chamber was varied between 1000 ng/ml and 20 μg/ml (corresponding to fivefold and 100-fold peak therapeutic serum levels, respectively). Because of its poor solubility in the buffer solution, the glyburide was first dissolved into 100 µl of ethanol and then added to the perfusate. A concentration of 200 ng/ml carbon 14-antipyrine was added to either the fetal or the maternal reservoir, again depending on the direction of transport being tested in each particular experiment. The pH of both circuits was continuously monitored and maintained at

7.4 by adjusting the carbon dioxide flow and concentration in the respirators. The temperature of both systems was maintained at 37° C by automatic temperature probe. Samples of 1.0 ml were taken from both reservoirs at the beginning of the experiment, at 10-minute intervals for the first hour, and every 30 minutes thereafter. The samples were immediately centrifuged and 0.4 ml aliquots taken for scintillation spectrometry. The remaining aliquots were frozen at -30° C for future analysis.

High-performance liquid chromatographic analysis. This analysis is a modification of that described by Emilsson et al. ¹⁰ The mobile phase consisted of a 50:50 mixture of 0.01 mol/L sodium phosphate buffer/acetonitrile titrated with phosphoric acid to pH 3.5. A Beckman C8, 5 μ m, 4.6 \times 250 mm column was used. Flow rate was maintained at 1.6 ml/min with an LDC/Milton Roy Constametric III metering pump, and absorption at 225 nm was determined with an LDC/Milton Roy model 3000 spectrometer. The glyburide peak was standardized with unlabeled glyburide (Sigma Chemical Co.). With this analysis the glyburide peak occurred at 7.5 minutes.

Liquid scintillation. The Beckman LS6800 liquid scintillation spectrometer was used to determine the deteriorations per minute of both tritium and carbon 14 in each sample, corresponding to glyburide and antipyrine, respectively. Concentrations of both glyburide and antipyrine were calculated from the deteriorations per minute obtained from each sample as a percentage of that obtained in the known original concentrations.

Metabolism. The metabolism of glyburide was determined by high-performance liquid chromatographic fractionation of the initial and final maternal and final fetal samples. The deteriorations per minute of each fraction were determined by liquid scintillation spectrometry, and the metabolism was calculated as a percentage on the basis of the difference in the deteriorations per minute of the glyburide peak fractions between the initial and final samples.

Placental uptake. When each perfusion was completed, the cotyledon was injected with a solution of malachite green through the fetal arterial catheter, thus staining the perfused portion of the cotyledon. The stained portion was excised and weighed. The entire cotyledon was then homogenized in distilled water and incubated for 1 hour in a 50:50 mixture of 30% hydrogen peroxide/70% perchloric acid to clarify the homogenate. Milliliter aliquots were then taken for scintillation spectrometry. The amount of uptake per volume of placenta was calculated from the deteriorations per minute in the homogenate, with the original donor sample used as the reference.

Diazepam and glyburide share similar protein binding, partition coefficients, and molecular weights. Be-

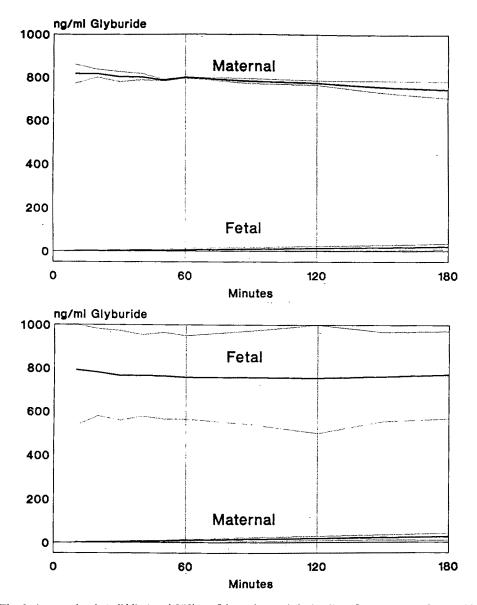


Fig. 1. Average levels (solid line) and 95% confidence interval (broken lines) for transport of glyburide in maternal-to-fetal (upper graph) and fetal-to-maternal (lower graph) directions.

cause of this, carbon 14-diazepam was added to several of the experiments in concentrations identical to those of glyburide to compare their transport. In these experiments unlabeled antipyrine was used, and its transport was determined by high-performance liquid chromatographic assay.11 Diazepam and glyburide concentrations were determined by liquid scintillation spectrometry in these experiments.

Equilibrium dialysis and partition coefficient. Equilibrium dialysis of glyburide and diazepam was performed across Spectrapore dialysis tubing with a molecular weight cutoff of 12,000 to 14,000 using Krebs-Ringer buffer with and without 2.0 gm/dl albumin to test their protein binding. A drug concentration of 1000 ng/ml was added to the buffer/albumin side, and the percent binding was determined by scintillation spectrometry after a 2-hour incubation period in a water bath at 37° C. A partition coefficient was determined by incubation and agitation in an octanol/buffer interface for 1 hour at 37° C, and again the differential concentrations were determined by scintillation spectrometry.

Statistics. The percentage of glyburide transported at 2 hours in both maternal-to-fetal and fetal-to-maternal directions was compared with the Mann-Whitney U test, and a p value of ≤ 0.05 was considered significant.

Results

The purity of the tritiated glyburide was 90%, and the compound remained stable in the Krebs-Ringer buffer during the 4-hour test period. There was vir-

Table I. Maternal-to-fetal transport of glyburide

Experiment No.	Maternal concentration (ng/ml)	Albumin concentration (gm/dl)	Cotyledon weight (gm)	Fetal concentration at 2 hr (ng/ml)	Glyburide/antipyrine transfer ratio	Transport (%)
1	1000	2.0	9.9	10.1	0.05	0.47
2	1000	2.0	9.5	23.4	0.05	1.10
3	1000	2.0	7.0	6.5	0.12	0.29

Table II. Fetal-to-maternal transport of glyburide

Experiment No.	Fetal concentration (ng/ml)	Albumin concentration (gm/dl)	Cotyledon weight (gm)	Maternal concentration at 2 hr (ng/ml)	Glyburide/antipyrine transfer ratio	Transport (%)
4	1000	2.0	14.4	19.8	0.02	0.57
5	1000	2.0	16.7	27.6	0.01	1.39
6	1000	2.0	12.0	8.9	0.04	0.72

Table III. Maternal-to-fetal transport of glyburide and diazepam

Experiment No.	Maternal concentration (ng/ml)	Albumin concentration (gm/dl)	Cotyledon weight (gm)	Fetal concentration at 2 hr (ng/ml)	Drug/antipyrine transfer ratio	Transport (%)
7	Glyburide 1,000	0.2	22.2	39.4	0.01	1.87
8	Glyburide 20,000	2.0	8.3	852.0	0.01	2.11
9	Glyburide 1,000			39.5	0.08	1.36
	, ,	2.0	48.8			
	Diazepam 1,000			543.2	4.12	72.14
10	Glyburide 1,000			52.1	0.15	2.17
	·	2.0	14.0			
	Diazepam 1,000			296.6	2.30	33.78

tually no transport of glyburide in either the maternal-to-fetal or the fetal-to-maternal direction. The average transport at 2 hours was 0.62% (n=3) from maternal-to-fetal circuits. The fetal-to-maternal transport was similar (p=0.35) at 0.89% (n=3). The overall transport of glyburide in both the maternal-to-fetal and fetal-to-maternal direction is illustrated in Fig. 1. The maternal-to-fetal transport of glyburide achieved an average fetal concentration of 26 ng/ml at 2 hours, when the original maternal concentration was 1000 ng/ml. This maternal concentration represents three-fold to eightfold therapeutic peak levels after a 5 mg oral dose in humans.

Varying the albumin concentration of both fetal and maternal perfusates had little effect on the transport of glyburide. Increasing the glyburide concentration to 100 times therapeutic levels likewise did not alter transport appreciably. A summary of these data is given in Tables I to III. There were no appreciable levels of metabolized glyburide detected in either donor or receiver perfusates or in the placental homogenates.

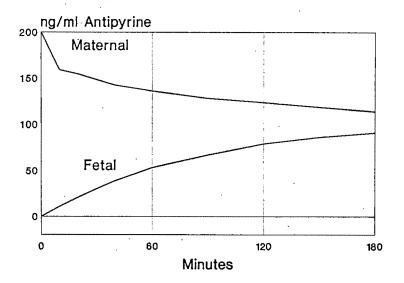
Placental uptake of glyburide ranged from 121 to 434 ng/gm placenta. This yielded a concentration of

glyburide per placental volume identical to the concentration in the perfusate solution in the receiving side of the experiment.

The observed transport of antipyrine was as expected. The antipyrine concentrations equilibrated between the maternal and fetal perfusates at approximately 120 minutes in both the maternal-to-fetal and fetal-to-maternal direction. The average maternal-to-fetal transport of both antipyrine and diazepam is illustrated in Fig. 2.

Equilibrium dialysis demonstrated both glyburide and diazepam to be 97% to 98% protein bound. Both substances yielded an octanol/buffer partition coefficient of 6.6.

The transport of diazepam in these experiments is characterized by rapid placental uptake and equilibration of fetal and maternal concentrations by 60 minutes. Maternal-to-fetal transport at 120 minutes ranges from 33% to 72%, and placental concentration is equivalent to both maternal and fetal concentration. This transport achieves an average fetal concentration of 420 ng/ml at 2 hours with the original maternal concentration of 1000 ng/ml.



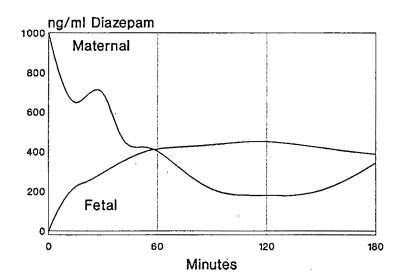


Fig. 2. Average transport of antipyrine (upper graph) and diazepam (lower graph) in maternal-to-fetal direction.

Comment

Our study is significant in that it is the first demonstration of negligible human placental transfer of glyburide. The implication from these findings is that maternally administered glyburide in pharmacologic dosages, even those greatly exceeding therapeutic levels, may not reach the fetus.

The recirculating single-cotyledon human placental model is used extensively to characterize the transport and metabolism of numerous pharmacologic agents and nutrients. 12, 13 It is well established as a safe in vitro surrogate for human placental transfer and is a desirable model because it allows study in the intact human placenta independent of any fetal metabolism. Another attribute of this system is the ability to validate each experiment individually with the addition of antipyrine. Antipyrine is transferred across the human placenta by simple diffusion in both maternal-to-fetal and fetal-to-maternal directions. With its use each experiment can be individually validated for proper transport characteristics and equilibration of antipyrine between the maternal and fetal systems.

On the basis of our study, placental transfer of substances cannot be predicted on the basis of protein binding, lipid solubility, or molecular weight alone. The comparison of the transport of glyburide and diazepam in our study demonstrates how dissimilar molecular configuration can cause a profound difference in the rate of transport of substances in spite of great similarity in the physical properties mentioned. We speculate that it is the rigidity of its tertiary structure that limits the transport of glyburide across the placental

membrane. This could be confirmed by comparing its transport and tertiary structure with those of the other sulfonylureas.

The findings in this study show (1) that glyburide does not cross the human placenta from the maternal to the fetal circulation in significant amounts, (2) that neither drug nor albumin concentration affects the rate of its transfer, (3) that glyburide is neither metabolized nor sequestered by the placenta, and (4) that the fetal-to-maternal transport of the drug is similar to the maternal-to-fetal transport; that is, it is not directionally selective and thus is suggestive of passive transfer.

Although several centers in other countries have reported the use of oral hypoglycemic agents during pregnancy,14-16 concern exists in the United States as to their possible effects on the developing fetus and newborn, on the basis of the assumption that significant transfer occurs across the placenta. These concerns have generally fallen into three categories: (1) the possible teratogenicity early in development, (2) the possible induction of fetal macrosomia by direct stimulation of insulin release from the fetal pancreas, and (3) the possibility of neonatal hypoglycemia if the newborn acquires pharmacologic levels. Our study demonstrates that glyburide does not cross the placenta in significant quantities; therefore the underlying assumption of these concerns is now brought into question. Our findings suggest the potential safety of this drug in pregnancy; however, the question of its ability to maintain adequate glycemic control remains to be answered. Previous studies have not addressed this issue.

Some studies have suggested a possible link between the use of oral hypoglycemics and fetal anomalies, fetal macrosomia, and neonatal hypoglycemia, whereas others have demonstrated no such relationship. 14-16 These aberrations are associated with poor maternal glycemic control and, in these studies, could have been the result of chronic maternal hyperglycemia rather than the effect of the agent itself. The majority of these studies are small and do not account for the level of maternal glycemic control, which could explain the conflicting results.

We have now demonstrated that glyburide does not cross the human placenta in significant amounts, so that the question of its safety from the standpoint of the fetus is greatly diminished and its use in the management of gestational diabetes becomes a possibility. We hypothesize that this agent may be a safer and more convenient alternative to insulin injections in selected

pregnant diabetic women, and we hope that our study will encourage further investigation into the use of oral hypoglycemics in pregnancy.

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Neutrophil attractant/activating peptide-1/interleukin-8 in term and preterm parturition

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The neutrophil is the leukocyte most frequently recruited into the amniotic fluid in cases of microbial invasion of the amniotic cavity. Neutrophil attractant/activating peptide-1/interleukin-8 is a newly identified cytokine that is capable of inducing selective neutrophil chemotaxis and activation. The purpose of this study was to examine the relationship between amniotic fluid concentrations of neutrophil attractant/activating peptide-1/interleukin-8, microbial invasion of the amniotic cavity, and parturition (term and preterm). Amniotic fluid neutrophil attractant/activating peptide-1/interleukin-8 was measured with an immunoassay validated for human amniotic fluid (sensitivity, 0.3 ng/ml). Fluid was obtained from women in the following groups: midtrimester (n = 38), term not in labor (n = 38), term in active labor (n = 67), and preterm labor with intact membranes (n = 62). Fluid was cultured for aerobic and anaerobic bacteria and Mycoplasma. Sterile amniotic fluid from most women in the midtrimester of pregnancy and women at term not in labor did not contain immunoreactive neutrophil attractant/activating peptide-1/interleukin-8. Microbial invasion of the amniotic cavity was associated with increased concentrations of neutrophil attractant/activating peptide-1/interleukin-8. The amniotic fluid of women with preterm labor and sterile amniotic fluid who had preterm delivery contained higher neutrophil attractant/activating peptide-1/interleukin-8 levels than did the amniotic fluid of women who responded to tocolysis and had delivery at term. Term parturition is associated with increased concentrations of neutrophil attractant/activating peptide-1/interleukin-8 in the amniotic fluid. We conclude that neutrophil attractant/activating peptide-1/interleukin-8 is part of the host response to microbial invasion of the amniotic cavity and that increased amniotic fluid availability of this cytokine occurs in term and preterm parturition. (Am J OBSTET GYNECOL 1991;165:813-20.)

Key words: Neutrophil attractant/activating peptide-1, interleukin-8, chemotaxis, chorioamnionitis, parturition, labor, prematurity, cytokines, amniotic fluid, preterm labor

Although the neutrophil is the leukocyte most frequently recruited into the amniotic fluid in cases of microbial invasion of the amniotic cavity, the mechanisms responsible for neutrophil chemotaxis in intrauterine infection have not been defined. Neutrophil chemoattractants include bacterial peptides, platelet activating factor, leukotriene B₄, and complement C5_a. Neutrophil attractant/activating peptide-1 (NAP-1)/interleukin-8 (IL-8) is a novel 8400 d cytokine that induces neutrophil chemotaxis and granule release. Originally purified from conditioned media obtained from bacterial endotoxin (lipopolysaccharide-

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LPS)—stimulated human mononuclear cells,^{5. 6} NAP-1/IL-8 was subsequently isolated from lipopolysaccharide-stimulated lung macrophages,⁷ mitogen-stimulated lymphocytes, and virus-infected fibroblasts.⁸ The NAP-1/IL-8 gene is placed at position 4q12-q21 on the human genome in a gene cluster with other members of the platelet factor-4 gene superfamily.⁹ The structure and biologic functions of NAP-1/IL-8 recently were reviewed.¹⁰

The studies described in this communication were undertaken to determine the relationship between gestational age, parturition (term and preterm), microbial invasion of the amniotic cavity, and amniotic fluid concentrations of NAP-1/IL-8.

Material and methods

Study design. A cross-sectional study was constructed according to the results of amniotic fluid cultures, gestational age, and labor status. Four groups were identified for study purposes. Group 1 comprised women in the midtrimester of pregnancy (gestational age, 16 to 18 weeks) undergoing amniocentesis for genetic indications (maternal age >35 years) (n=38). Group 2

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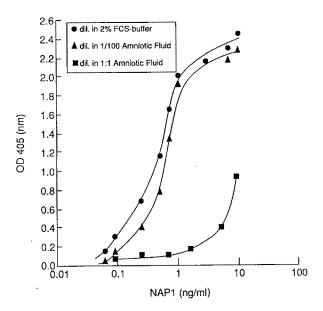


Fig. 1. Validation of immunoassay for detection of NAP-1/IL-8 in human amniotic fluid. Assay standard curve was constructed by adding increasing concentrations of human recombinant NAP-1/IL-8 to assay buffer containing 2% fetal calf serum (FCS) (circles). Poor recovery was observed when human recombinant NAP-1/IL-8 was added to pure pooled human amniotic fluid (squares). Parallelism was achieved when amniotic fluid was diluted (1:100) with assay buffer and 2% fetal calf serum (triangles). Vertical axis displays optical density whereas horizontal axis illustrates final concentrations of human recombinant NAP-1/IL-8.

was composed of women at term (38 to 39 weeks) who had amniocentesis for the assessment of fetal lung maturity before elective cesarean section (n = 38). Group 3 was made up of women in spontaneous active labor at term (38 to 41 weeks) (n = 67). These patients were subclassified according to the results of amniotic fluid culture into subgroups: subgroup 3a, women with negative amniotic fluid cultures (n = 49); subgroup 3b, women with positive amniotic fluid cultures (n = 18). Group 4 consisted of women admitted with preterm labor and intact membranes (n = 62). This group was divided into three subgroups according to amniotic fluid culture results and response to tocolysis: subgroup 4a, women with negative amniotic fluid cultures who responded to tocolysis and delivered at term (n = 25); subgroup 4b, women with negative amniotic fluid cultures and delivery of a preterm neonate in spite of tocolysis (n = 24); subgroup 4c, women with positive amniotic fluid cultures and premature delivery (n = 13).

Nitrazine, pooling, and ferning tests were performed in all women in labor to exclude rupture of membranes. Preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 minutes associated with changes in the Bishop score. Ritodrine was administered intravenously as the tocolytic agent according to a protocol described else-

where by Caritis. Amniocentesis was performed before the initiation of therapy. Failure of tocolysis was diagnosed when cervical dilatation progressed beyond 5 cm or delivery occurred.

Retrieval of amniotic fluid. Amniotic fluid was retrieved by transabdominal amniocentesis. Amniocentesis for microbiologic studies is offered in our institution to all patients with the diagnosis of preterm labor. Women in active labor at term were identified by searching our obstetric data base to identify patients who underwent amniocentesis because of suspected preterm labor but who subsequently delivered a term neonate (within 12 hours of amniocentesis). The identification numbers of patients fulfilling these criteria were cross-referenced with samples stored in our amniotic fluid bank. This bank consists of aliquots of amniotic fluid that remain after clinically indicated tests are performed. For the purposes of storage in this bank, fluid was centrifuged at 200g for 10 minutes at 4° C and stored in polypropylene tubes at -70° C until assayed.

Microbiologic culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after collection. Plating occurred within 30 minutes of collection in all cases. Fluid was cultured for aerobic and anaerobic bacteria and for *Mycoplasma* according to methods previously described.¹²

Quantitation of NAP-1/IL-8. NAP-1/IL-8 was measured with an enzyme-linked immunoassay. The characteristics of this assay have been recently described.13 The sensitivity of the assay in assay buffer is 3 pg/ml, and validation of its use for human amniotic fluid was performed. Fig. 1 illustrates that dilution of the fluid (1:100) was required to achieve parallelism between the standard curve in buffer and in amniotic fluid. The sensitivity of the assay in amniotic fluid was 0.3 ng/ml. The mean intraassay coefficient of variation was determined by assaying 28 replicates of three amniotic fluid samples with detectable NAP-1/IL-8 in the same assay. The mean, standard deviation, and percent coefficient of variation were as follows: For sample 1, mean = 1.6 ng/ml, SD = 0.3 ng/ml, and coefficient of variation = 22%. For sample 2, mean = 50.3 ng/ml, SD = 5.2 ng/ml, and coefficient of variation = 10.3%. For sample 3, mean = 106.4 ng/ml, SD = 9.2 ng/ml, and coefficient of variation = 8.7%. The interassay coefficient of variation was 10.9% (mean of three different assays).

Amniotic fluid absolute neutrophil count. Amniotic fluid white blood cell counts and differential counts were performed in a set of patients with preterm labor and intact membranes. However, this test was performed only rarely in the other study groups. The method used for the amniotic fluid white blood cell count has been recently described.¹⁴

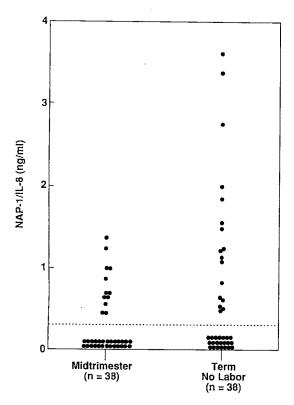


Fig. 2. Amniotic fluid concentrations of NAP-1/IL-8 in women in midtrimester of pregnancy and in women at term not in labor with negative amniotic fluid cultures for microorganisms. Interrupted horizontal line corresponds to sensitivity of assay (0.3 ng/ml). For midtrimester, median = 0 and range = 0to 1.35 ng/ml. For term no labor, median = 0 and range = 0to 3.62 ng/ml. There was no significant difference in median or proportion of women with detectable NAP-1/IL-8 between these two groups. However, proportion of patients with NAP-1/IL-8 concentrations above 1 ng/ml was significantly greater in women at term not in labor (p < 0.01).

Statistical analysis. Comparisons between proportions were performed with either a χ^2 test or Fisher's exact test. A Kruskal-Wallis analysis of variance was used to compare amniotic fluid NAP-1/IL-8 concentrations and absolute neutrophil counts among study groups. Dunn's test was used for multiple post hoc comparisons. A Spearman rank correlation test was used to examine the relationship between the amniotic fluid absolute neutrophil count and amniotic fluid NAP-1/IL-8 concentrations.

Results

Most amniotic fluid samples from women in the midtrimester of pregnancy and at term not in labor did not have detectable immunoreactive NAP-1/IL-8 (Fig. 2). However, the proportion of amniotic fluid samples with NAP-1/IL-8 concentrations above 1 ng/ml was significantly greater in women at term not in labor than in women in the midtrimester of pregnancy (64.7%

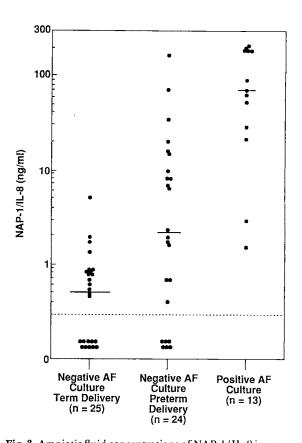


Fig. 3. Amniotic fluid concentrations of NAP-1/IL-8 in women with preterm labor and intact membranes. Interrupted horizontal line corresponds to sensitivity of assay (0.3 ng/ml). For women with negative amniotic fluid cultures and term delivery, median = 0.5 ng/ml and range = 0 to 5.1 ng/ml. For women with negative amniotic fluid cultures and delivery of preterm neonates, median = 2.1 ng/ml and range = 0 to 162 ng/ml. For women with positive amniotic fluid cultures and delivery of preterm neonates, median = 70 ng/ml and range = 1.5 to 203 ng/ml. By Kruskal-Wallis analysis of variance, H = 29.2, p < 0.0001. Patients with positive amniotic fluid cultures had higher median amniotic fluid NAP-1/IL-8 levels than did those in the other two groups (p < 0.05). Patients with negative amniotic fluid cultures and lack of response to tocolysis had significantly higher median NAP-1/IL-8 concentrations than did patients with negative amniotic fluid cultures who responded to tocolysis and had term delivery (p < 0.05).

[11/17] vs 16.6% [2/12]; p < 0.01). All patients in these two groups had negative amniotic fluid cultures for microorganisms.

Fig. 3 illustrates NAP-1/IL-8 concentrations in the amniotic fluid of women with preterm labor and intact membranes. All patients with positive amniotic fluid cultures for microorganisms had detectable amniotic fluid NAP-1/IL-8. Furthermore, these patients had significantly higher amniotic fluid NAP-1/IL-8 levels than did patients with negative amniotic fluid cultures (p < 0.05). Patients with negative amniotic fluid cultures who were unresponsive to tocolysis and had delivery of preterm neonates had significantly higher me-

Table I. Clinical data of patients with	preterm labor and microbial invasion of amniotic cavity
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No.	Bacteria on Gram stain	Culture	Placental pathologic condition	Amniotic fluid white blood cell count (No.!mm³)	Amniotic fluid NAP-1/IL-8 (ng/ml)
1	· +	Fusobacterium sp.	Chorioamnionitis	1100	202.5
2	+	Capnocytophaga (Chorioamnionitis	56	190.4
		Clostridium sp.			
3	+ .	Fusobacterium sp.	N/A	835	189.3
		Mycoplasma hominis Ureaplasma urealyticum			
4	_	Ureaplasma urealyticum	Chorioamnionitis	1100	64.4
5	_	Streptococcus agalactiae	N/A	1	1.5
6	+ .	Bacteroides fragilis	Chorioamnionitis	162	186
_		Fusobacterium sp.			
7	+	Candida sp.	Chorioamnionitis	_	52.5
8	_	Ureaplasma urealyticum	N/A	502	28.1
9		Ureaplasma urealyticum	Chorioamnionitis	580	20.6
10	· +	Fusobacterium sp.	N/A	260	70
11	+	Fusobacterium sp.	Chorioamnionitis		203
12	_	Ureaplasma urealyticum	_	162	3
13	+	Fusobacterium sp.	Chorioamnionitis	- ,	92.4
		Mycoplasma hominis Ureaplasma urealyticum	•		

N/A, Not available.

dian amniotic fluid NAP-1/IL-8 concentrations than did those who responded to tocolysis (p < 0.05).

Table I illustrates the clinical data, microbiologic characteristics, NAP-1/IL-8 concentrations, and placental pathologic features in women with positive amniotic fluid cultures. The most common microbial isolates were *Ureaplasma urealyticum* (n=6) and *Fusobacterium* sp. (n=6). Polymicrobial infections were detected in 30.7% (4/13) of cases, and in 30.7% (4/13) the only isolates were *Mycoplasma*. All patients in whom the placentas were examined had histologic evidence of chorioamnionitis. None of the patients had clinical evidence of chorioamnionitis, and there were no cases of documented neonatal sepsis.

To explore the relationship between amniotic fluid NAP-1/IL-8 immunoreactivity and biologic activity, we examined placental histopathologic characteristics, the amniotic fluid white blood cell count, and amniotic fluid NAP-1/IL-8 levels. Pathologic examination of the placenta was performed in 22 of the 37 patients with preterm delivery. All patients with histologic chorioamnionitis (n=12) had detectable NAP-1/IL-8, whereas 40% (4/10) of those without acute inflammatory lesions of the placenta had detectable amniotic fluid NAP-1/IL-8. The amniotic fluid concentrations of this cytokine were significantly higher in women with histologic chorioamnionitis than in women without acute inflammatory lesions of the placenta (Fig. 4).

Fig. 5 displays amniotic fluid absolute neutrophil counts in patients with preterm labor. It is apparent that the data are similar to amniotic fluid IL-8 concentrations in these patients (Fig. 3). Women with positive amniotic fluid cultures had significantly greater abso-

lute amniotic fluid neutrophil counts than did women with negative amniotic fluid cultures in the other two groups. Although patients who were unresponsive to tocolysis and had negative cultures had higher amniotic fluid neutrophil counts than did women with negative cultures and delivery at term, this difference fell short of reaching conventional significance (p=0.07). There was a significant correlation between amniotic fluid neutrophil count and amniotic fluid NAP-1/IL-8 levels (r=0.44, p<0.001, Spearman rank correlation), but no correlation between cervical dilatation and amniotic fluid NAP-1/IL-8 concentrations (p>0.05, Spearman rank test).

Fig. 6 displays the amniotic fluid NAP-1/IL-8 concentrations of women at term. The amniotic fluid of women in active labor with negative amniotic fluid cultures had significantly higher NAP-1/IL-8 concentrations than did the amniotic fluid of women not in labor. Moreover, in women with positive amniotic fluid cultures concentrations of NAP-1/IL-8 were higher than in women at term in labor. There was no relationship between cervical dilatation and amniotic fluid concentrations of NAP-1/IL-8 (p > 0.05, Spearman rank test).

Table II displays the microbiologic characteristics and amniotic fluid NAP-1/IL-8 levels in women with microbial invasion of the amniotic cavity in term labor. The most common microbial isolate was Ureaplasma urealyticum (n = 11). Polymicrobial infections were detected in 11.1% (2/18) of cases, and in 50% (9/18) the only isolates were Mycoplasma.

Finally, the amniotic fluid of women with preterm labor and positive amniotic fluid cultures had a higher median NAP-1/IL-8 than that of the amniotic fluid of

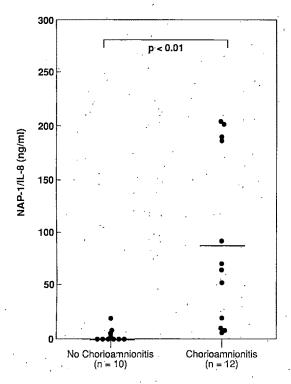


Fig. 4. Amniotic fluid NAP-1/IL-8 concentrations in women with preterm labor and intact membranes who subsequently had delivery of preterm neonates according to placental histopathologic results. In patients with histologic chorioamnionitis amniotic fluid NAP-1/IL-8 values were significantly higher than in women with negative amniotic fluid cultures (for chorioamnionitis, median = 67.8 ng/ml and range = 1.5 to 203 ng/ml; for no chorioamnionitis, median = 0.5 ng/ml and range = 0 to 20.3 ng/ml; p < 0.001, Mann-Whitney U test).

women in labor at term with positive amniotic fluid cultures (for preterm labor, median = 70 ng/ml and range = 1.5 to 203 ng/ml; for term labor, median = 4.8 ng/ml and range = 0.41 to 98.8 ng/ml; p < 0.001, Mann-Whitney *U* test).

Comment

The major finding of this study is that parturition is associated with increased amniotic fluid concentrations of NAP-1/IL-8. This was clearly the case in spontaneous term labor where women with sterile amniotic fluid had a higher median amniotic fluid concentration of NAP-1/IL-8 than did women not in labor at term (Fig. 6). In addition, the median amniotic fluid NAP-1/IL-8 concentration was higher in patients in preterm labor with negative amniotic fluid cultures who progressed to preterm delivery than in patients with preterm labor who responded to tocolysis (Fig. 3). Therefore an elevation in amniotic fluid NAP-1/IL-8 concentrations seems to be a phenomenon associated with labor progressing to delivery. The physiologic role of amniotic fluid NAP-1/IL-8 in parturition has not been determined. Since parturition has been likened

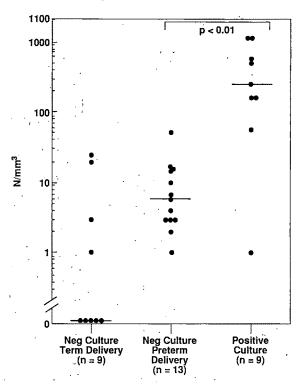


Fig. 5. Neutrophil amniotic fluid count according to amniotic fluid culture results and response to tocolysis of patients with NAP-1/IL-8 determinations in amniotic fluid. Vertical axis displays neutrophils per cubic millimeter. For women with negative amniotic fluid cultures and delivery at term, median = 0 cells/mm³ and range = 0 to 25 cells/mm³. For women with negative amniotic fluid cultures and delivery of preterm neonates, median = 6 cells/mm³ and range = 1 to 50 cells/mm³. For women with positive amniotic fluid cultures and delivery of preterm neonates, median = 260 cells/mm³ and range = 1 to 1100 cells/mm³. By Kruskal-Wallis analysis of variance, H = 15.7; p < 0.0001. Patients with positive amniotic fluid cultures had higher median absolute neutrophil counts than did those in the other two groups (p < 0.01 and p < 0.05). However, difference between absolute amniotic fluid neutrophil counts of patients with negative amniotic fluid cultures and lack of response to tocolysis and those of patients with negative amniotic fluid cultures, response to tocolysis, and delivery at term fell short of reaching significance (p = 0.07).

to an inflammatory process,15 NAP-1/IL-8 may be a signal for neutrophil recruitment and activation in reproductive tissues during labor.

Another major finding of this study is that microbial invasion of the amniotic cavity is associated with an elevation of amniotic fluid NAP-1/IL-8 concentrations. This elevation was dramatic in women with preterm labor and intact membranes (Fig. 3). Since NAP-1/IL-8 is produced by activated macrophages in response to bacterial products⁵⁻⁷ and is an important inflammatory mediator, it is not surprising to find it in such high concentrations in the setting of microbial invasion of the amniotic cavity. The microorganism isolated most frequently from amniotic fluid was Urea-

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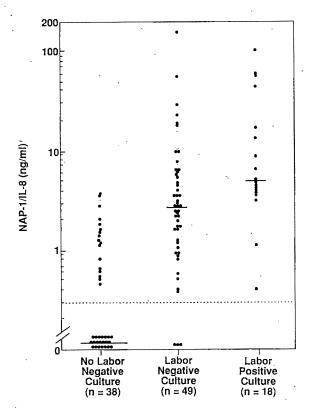


Fig. 6. Amniotic fluid concentrations of NAP-I/IL-8 in women at term. Interrupted horizontal line corresponds to sensitivity of assay (0.3 ng/ml). For women not in labor, median = 0 ng/ml and range = 0 to 3.6 ng/ml. For women in labor with negative amniotic fluid cultures, median = 2.68 ng/ml and range = 0 to 152 ng/ml. For women in labor with positive amniotic fluid cultures, median = 4.8 ng/ml and range = 0.4 to 98.8 ng/ml. By Kruskal-Wallis analysis of variance, H = 47.4; p < 0.0001. Patients with spontaneous labor had higher median amniotic fluid NAP-1/IL-8 concentrations than did women not in labor (p < 0.05). Patients in labor with positive amniotic fluid cultures had higher median amniotic fluid NAP-1/IL-8 concentrations than did women in labor with negative amniotic fluid cultures (p > 0.05 but < 0.10).

plasma urealyticum. This observation is consistent with previous reports from our institution in which this microorganism was the most common isolate from the amniotic fluid of women with preterm premature rupture of membranes or with preterm labor and intact membranes. We have recently demonstrated that women with preterm labor and intact membranes with positive amniotic fluid cultures for *Ureaplasma urealyticum* have a shorter amniocentesis-to-delivery interval than women with sterile amniotic fluid. Moreover, the data presented in this report suggest that microbial invasion of the amniotic cavity with this microorganism is associated with a significant host response as measured by the concentration of NAP-1/IL-8 in the amniotic fluid.

We have no direct evidence for the biologic activity of immunoreactive amniotic fluid NAP-1/IL-8. Such evidence would require purification of NAP-1/IL-8 from amniotic fluid and demonstration of its biologic activity with a bioassay. However, our data indicate a correlation between amniotic fluid concentrations of NAP-1/IL-8 and in vivo indices of inflammation. For example, amniotic fluid NAP-1/IL-8 concentrations were significantly higher in patients with histologic chorioamnionitis than in patients without acute inflammatory lesions of the placenta (Fig. 4). Moreover, there was a correlation between amniotic fluid NAP-1/IL-8 concentrations and amniotic fluid absolute neutrophil count. These data support a role for NAP-1/IL-8 in the recruitment of neutrophils to the maternal-fetal interface and amniotic cavity in the setting of intraamniotic infection. Our results are similar to the observations made by Pankuch et al.16 with the use of an in vitro leukotaxis bioassav.

The magnitude of the increase in amniotic fluid NAP-1/IL-8 concentrations in the setting of microbial invasion of the amniotic cavity was related to gestational age. The median amniotic fluid NAP-1/IL-8 concentration was fourteenfold higher in women with preterm labor and positive amniotic fluid cultures than in women with term labor and positive amniotic fluid cultures. The explanation for this finding is not readily apparent. We explored the possibility that differences in qualitative microbiologic characteristics might be responsible for these observations. However, the proportion of patients with polymicrobial invasion of the amniotic cavity and positive cultures limited to Mycoplasma or with gram-negative microorganisms was not different between the preterm labor and term groups (isolated Mycoplasma: 30.7% [4/13] vs 50% [9/18]; polymicrobial: 30.7% [4/13] vs 11.1% [2/18]; positive cultures with gram-negative microorganisms: 23% [6/13] vs 16.6% [3/18]; all p values >0.05, Fisher's exact test). Although it is possible that the inoculum size might be different between groups, we were unable to test this hypothesis because the majority of patients did not have quantitative microbiologic evaluation. An alternative view is that the concentration of NAP-1/IL-8 in amniotic fluid is dependent on the pathway of microbial invasion and the time allowed for microbe-host interaction before amniocentesis. Amniotic fluid NAP-1/IL-8 is thought to be, at least partially, of decidual origin (Romero R. Unpublished observations), and therefore the state and duration of decidual activation may be critical to the secretion of NAP-1/IL-8 into the amniotic fluid. If microorganisms from the lower genital tract gain access to the amniotic cavity directly without a significant "decidual stage," NAP-1/IL-8 secretion into the amniotic fluid could be modest or even absent. This may be the course of events in term labor with microbial invasion of the amniotic cavity. On the other hand, if microorganisms gain ac-

Table II. Microbiologic results in patients	with microbial invasion o	of amniotic cavity in spontaneous labor
at term		·

No.	Bacteria on Gram stain	Culture	NAP-1/IL-8
1	<u> </u>	Ureaplasma urealyticum	56.41
2	_	Ureaplasma urealyticum	16.3
3		Ureaplasma urealyticum	43.82
4	_	Ureaplasma urealyticum	98.8
5	_	Ureaplasma urealyticum	12.84
6		Escherichia coli	3.51
		Ureaplasma urealyticum	
7		Ureaplasma urealyticum	4.98
8		Ureaplasma urealyticum	3.07
9		Peptostreptococcus +2	3.96
10		Streptococcus group G	4.77
11		Ureaplasma urealyticum	4.54
12	•	Lactobacillus (–)	6.41
13	· <u>-</u>	Streptococcus viridans (-)	0.41
14	_	Candida albicans	4.22
		Ureaplasma urealyticum	•
15	_	Gram-negative, not identified	59.0
16	+	Streptococcus agalactiae	8.57
17	_	Ureaplasma urealyticum	3.67
18	+	Gram-negative, not identified	1.08

cess to the decidua for a sufficient time, NAP-1/IL-8 secretion by activated macrophages may lead to significant accumulation of this cytokine in the amniotic cavity. We propose that this is the likely course of events in premature labor with intact membranes.

The demonstration of the presence of NAP-1/IL-8 in the amniotic cavity is of considerable biologic importance because this peptide is an inflammatory mediator and enhances host defense against infection. In the uterus the first cellular line of defense against infection is probably resident decidual macrophages. Activation of these cells by microbial products leads to NAP-1/IL-8 production (Romero R. Unpublished observations), which, in turn, may recruit neutrophils to the site of microbial invasion. Interleukin-1 and tumor necrosis factor are also produced by human decidua in response to bacterial products,17.18 and these two cytokines can induce IL-8 production by several cell types. 19-24 Thus decidua contains the cellular elements necessary for the existence of an amplification loop to ensure adequate recruitment and activation of neutrophils for host defense. During the course of parturition, tissue disruption and noninfectious inflammatory mediators may lead to secretion and recruitment of neutrophils. In this setting neutrophils may play an important role in clearing cellular debris after delivery of the placenta, thereby enabling normal uterine involution.

In conclusion, we have demonstrated NAP-1/IL-8 is part of the host response to microbial invasion of the amniotic cavity and that amniotic fluid concentrations of this cytokine increase during preterm and term parturition. Further studies are required to establish the sources, regulatory mechanism, and biologic functions of this cytokine in reproductive tissue. Studies are also required to determine the relative role of NAP-1/IL-8 in the intrauterine cytokine network.

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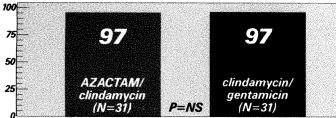
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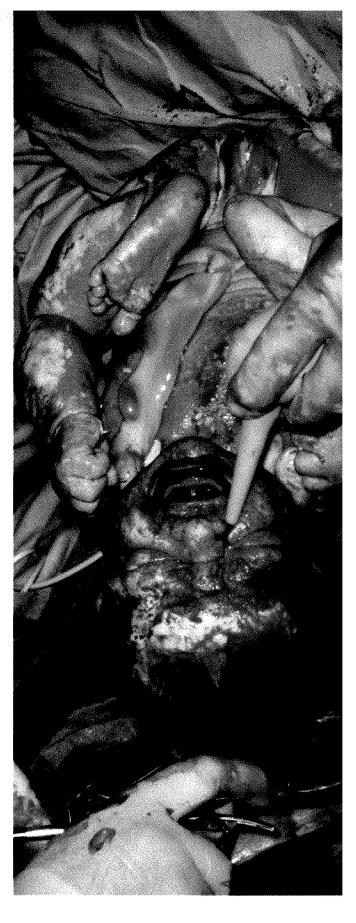


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INDICATIONS AND USAGE—Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: Urinary Tract Infections (conplicated), including pyelonephritis and cystitis (initial and recurrent) caused by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter species* and Serratia marcescens*. Lower Respiratory Tract Infections, including pneumonia and bronchitis caused by Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter species and Serratia marcescens*. Septicemia caused by Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens* and Enterobacter species Skin and Skin-Structure Infections, including those associated with postoperative wounds, ulcers and burns caused by Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter species*, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Citrobacter species* intra-abdominal Infections, including peritonitis caused by Escherichia coli, Klebsiella species, including K. pneumoniae, Enterobacter species* including E. cloacae*, Pseudomonas aeruginosa, Citrobacter species* including C. freundii* and Serratia species* including S. marcescens*. Gynecologic Infections, including endometritis and pelvic cellulitis caused by Escherichia coli, Klebsiella pneumoniae*, Enterobacter species* including E. cloacae* and Proteus mirabilis*.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gramnegative aerobic pathogens seen in general surgery.

Concurrent Therapy—Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organism(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an anti-anaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS—Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS—Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to lifethreatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin).

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS—General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility—Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed in vivo and in vitro with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy-Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers—Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use-Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS—Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity: Hypersensitivity—anaphylaxis, angioedema, bronchospasm. Hematologic—pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. Gastrointestinal—abdominal cramps; rare cases of C. difficile-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). Dermatologic—purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. Cardiovascular—hypotension, transient ECG changes (ventricular bigeminy and PVC). Respiratory—one patient experienced flushing, chest pain, and dyspnea. Hepatobiliary—hepatitis, jaundice. Nervous System—seizure, confusion, vertigo, paresthesia, insomnia, dizziness. Musculoskeletal—muscular aches. Special Senses—tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. Other—vaginal candidiasis, vaginitis, breast tenderness. Body as a Whole—weakness, headache, fever, malaise.

Adverse Laboratory Changes—Those reported without regard to drug relationship during clinical trials were: *Hepatic*—elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*—increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Henal*—increases in serum creatinine.

OVERDOSAGE—If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION-Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg**, or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231E)

References: 1. Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritic Clin Ther 10(11:36–39, 1987. 2. Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet

Gynecol 65:825–829, 1985. 3. Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703–S708, 1985.



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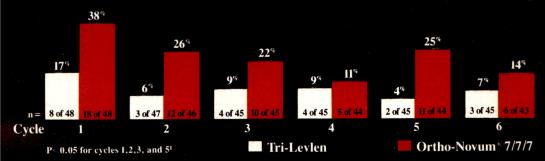
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Multicenter study demonstrates significantly less breakthrough bleeding/spotting through the first six cycles¹



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* Incidence defined as percentage of women with a bleeding event occurring at least once during a pill cycle. Serious as well as minor side effects have been reported with the use of oral contraceptives.

The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

† Defined as intermenstrual bleeding.

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TRI-LEVLEN® Levonorgestrel and ethinyl estradiol tablets-Triphasic regimen

TRI-LEVLEN® 28 TRI-LEVLEN® 21

BRIEF SUMMARY

BRIEF SUMMARYTri-Levien®—6 brown toblets, each containing 0.050 mg of levonorgestrel (d.-)-13 beta-ethyl-17-olpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17a-pregna-1.3.5(10)-trien-20-yne-3, 17-diol), 5 white toblets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol, 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception

Controlidections:

Croil confragetives should not be used in women with any of the following conditions: thrombophiebitis or thromboembolic disorders, a past history of deep-vein thrombophiebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estragendependent peoplosia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pitiuse, hepatic adenomas ar carcinomas, known or suspected pregnancy.

Marnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

who use and contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocordial infarction, thromboemboism, stroke, hepatic neoplosis, galibloader disease, and hyperfension, although the risk of serious morbidly or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and morbidity increases significantly in the presence of other underlying risk factors such as hyperfension, hyperipidemias, obesity and diabetes. Practition-ers prescribing and contraceptives should be familiar with the following information relating to these risk in incommon error process. The information contained in this package insert is principally based on studies carried out in patients who used and contraceptives with higher formulations of stripages and progestogens from the roleowing information relating to these risks of relating the contraceptive with lower formulations of both estragens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types, retrospective or case control studies and prospective or cord contraceptive users to that among nanusers. The relative risk of disease, namely, a ratio of the incidence of a disease among arabicontraceptive users and nanusers. The relative risk of disease, namely, a ratio of the incidence of a disease among arabicontraceptive users and nanusers. The entitubule risk does provide information about the actual ccinical occurrence of a disease. Contrative price and contraceptive users and nanusers. The entitubule risk does provide information about the actual ccinical occurrence of a disease. Although a propulation. For further information, the redder is referred to a text on epidemiological methods.

Intromboembolic Disorders and Other Vascular Problems

A Myocordial Infactant. An increased risk of myocordial infaction has been attributed to oral-contraceptive user. The risk is surpriorly in

- risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thramboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to ard contraceptives and related to length of use and disappears after pill use is stopped. A two-of todi increase in relative risk of postoperative thromboembolic complications has been reported with the use of and contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such moment who have predisposing conditions is wice that of women without such model conditions. If feasible, and contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, and contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination. pregnancy termination.
- pregnancy termination.

 c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of serebrovascular diseases. Oral contraceptives disease, and to be a risk factor for both users and nonusers for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes in a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The attributable risk is also greater in older women.

 A Dose-related risk of vascular disease from roal contraceptives. 18 for normotensive users on 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

 A Dose-related risk of vascular disease from roal contraceptives. A positive association has been observed between the amount of estragen and progestagen in and contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins (HDL) has been associated with an increased incidence of ischemic heard disease. Because estrogens and progestagen and the nature and absolute amount of progestagen are in the contraceptive. The amount of both hormones should be considered in the contraceptive. The amount of both hormones should be considered in the contraceptive amount of progestagen and the needs of the individual potent. New acceptors of oral-contraceptive agents should be storted on preparations containing less than 50 mcg of estragen.
- of estrogen.

 e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of and contraceptives. In a study in the United States, the risk of developing myocardial inforction after discontinuing, and contraceptives persists for all easily syears for women 40-49 years who had used and contraceptives for more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted of at least 6 years after discontinuation of and contraceptives, although excess risk was very small. However, both studies were performed with analysis of estrogens.
- cell estinguies.

 2. Estimates of Mortality from Contraceptive Use. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method folium. Each method of contraceptive methods plus the risk attributable to pregnancy in the event of method risk of death associated with contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth contral is less than that associated with childrinh. The observation of a possible increase in risk of mortality with age for oraccontraceptive users is based on data gathered in the 1970 s-but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of orac-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Moternal Health Turgs Advisory Committee was asked to review the topic in 1989. The Committee concluded that dithough cardiovascular disease with the use of oral contraceptive use enter age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are switch may be necessary if such women and on that over access to effective and acceptable means of contraceptive. Therefore, thick may be necessary if such women and on that over access to effective and acceptable means of contraception. Therefore the Committee recommended that the benefits of orai-contraceptive use by healthy nonsmoking women over 40 may autweigh the possible risks. Of course, older women, as all women who take oral contraceptives,
- is effective.

 3. Carpinana of the Reproductive Organs. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarion and servical cancer in women using and contraceptives. The overwhelming evidence in the literature suggests that use of and contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) states of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) states are sufficiently also showed no latent effect on the risk of developing press cancer, of though the methodology of the studies have shown a slightly increased relative risk of developing presst cancer, of though the methodology of the studies. Surjective studies suggest that and-contraceptive use has been associated with an increase in the risk of cervical intrapethietial nepilical or some populations of women. However, there continues to be controvery about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between ori-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

 A Hepatic Nepolosia. Bening hepatic adenomas are associated with ori-contraceptive use and the residence of bening the part of the relationship to the contracent of the relationship the part of the relationship to the contracent of the relationship to the relati
- 4. Hepatic Neoplasia. Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use, Rupture of rare, benign, hepatic across any acuse death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular.

carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users. 5. Ocular Lesions. There have been clinical case reports of retinal thrombosis associated with the use of oral controceptive Soral controceptives of the control through the con

- token immediately.

 6. Onto-Contraceptive Use Before or During Early Pregnancy Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used and contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives in induce withdrawal bleeding should not be used so test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any potient who has missed from consecutive periods, pregnancy should be considered that for any potient who has missed from consecutive periods, pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is continued.

 7. Gallbladder Disease Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive uses may be minimal. The recent findings of minimal tisk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.
- Infinition Continuing Jower normal abuses of earningers and projectogens.

 8. Carbohydrate and Lipid Metabolic Effects, Ordi controceptives have been shown to cause glucose intolerance in a significant percentage of users. Ordi controceptives containing greater than 75 micrograms of estragens cause hyperinsulinism, while lower doses of estragen cause less glucose intolerance. Progestagens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic women, and controceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while foking and controceptives. A small proportion of women with laves persistent hypertriplycenia while on the pill. As discussed earlier (see: "Womings" to and Id.), changes in serum triglycerides and lipoprotein levels have been reported in ordi-controceptive. As million of controceptive and the programment of the pill. n oral-contraceptive users.
- in oral-contraceptive users.

 9 Elevated Blood Pressure An increase in blood pressure has been reported in women taking and contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hyperfersions increases with increasing quantiles of progestogers. Women with a history of hyperfension or higher decisions, and increases in the increasing quantiles of contraception. If women with hyperfension elect to use and contraceptives, they should be encouraged to use another method of contraception. If women with hyperfension elect to use and contraceptives, they should be monitored closely, and if significant leviation of blood pressure occurs, and contraceptives should be disconfinued from solventing, elevated blood pressure will return to normal after stopping and contraceptives, and there is no difference in the occurrence of hyperfension among ever-and never-users. of hypertension among ever-and never-users.

of hypertension among ever-and never-users.

O Headache The onset or exocerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

Il Bleeding Irregularities Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestagen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal regional bleeding; if pothology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pall amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

post-pill amenormeo or oligomenormea, especially when such a condition was pressistent.

Procourtions

1. PHYSICAL EXAMINATION AND FOLLOW UP A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure breads, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted for uite out malignancy. Women with a strong farmily history of breads concer or who have breast nodules should be manifored with particular care 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be monitored with particular care 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be monitored with particular care 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias the control of hyperlipidemias more difficult (See "Wornings" to). J. LIVER PUNCTION. If jounded develops in any woman receiving such drugs, the medication should be discontinued. Steriot hormones may be poorly meribobilized in prients with imported fiver function. A FUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with coultion, and only with careful monitoring, in potients with conditions which might be aggravated by fluid retention. S. BMOTIONAL DISORDERS, Polientes becoming significantly depressed while toxing prot contraceptives should stop the medication and use an attempte method of contraceptive in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued

Information for the Patient See Patient Labeling.

Adverse Reactions

Adverse Reactions An increased risk of the following serious adverse reactions has been associated with the use of and contraceptives (see "Warnings' section): thrombophilebits, orteriol thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, galibladder disease, hepatic adenomas or benign liver tumors. There is evidence of an association between the following conditions and the use of anal contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving and contraceptives and are believed to be drug related nouses, worning adverse reactions have been reported in patients receiving and contraceptives and are believed to be drug related nouses, worning, gastromitestinal symptoms (such as obdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorable, temporary interfillity after discontinuation of treatment; edema, melasorable may presist, breast changes: tenderness, enlargement, secretion, change in weight (increase or decrease), change in cervical erosion and secretion, diminution in lactation when given immediately postpartum; cholestatic joundice; migraine; rask (allergic), mental depression, reduced folerance to carbohydrates, vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

tool lenses.

lowing adverse reachions have been reported in users of oral contraceptives and the association has been neither confirmed
uted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome,
chernousness, dizziness, hirsultism, loss of scolp had, eighterna multiforme, cerebral-vascular disease with mitral valve
se, lupus-like syndromes, enthema nodosum, hemormogic eruption, vagaintis, porphyria, impaired renal function, hemolytic
syndrome, Budd-Chlori syndrome, acne, changes in libido, colifis, sickle-cell disease.

Overdosage
Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children.
Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Overloosage may evous housed, and winnorwal obesing may occur in temales.

The following nancontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which tangely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestronol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency onemia, decreased incidence of dysmenorthea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence

of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer Dosage and Administration
To achieve maximum continues

Debugge and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Toblets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert.

60627-0 5/90

Reference: 1. Data on file, Berlex Laboratories.



Berlex Laboratories, Wayne, New Jersey 07470

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914300070

Amniotic fluid white blood cell count: A rapid and simple test to diagnose microbial invasion of the amniotic cavity and predict preterm delivery

Roberto Romero, MD, Ruben Quintero, MD, Jose Nores, MD, Cecilia Avila, MD, Moshe Mazor, MD, Shuichi Hanaoka, MD, Zion Hagay, MD, Lydia Merchant, MS, and John C. Hobbins, MD

New Haven, Connecticut

The purpose of this study was to determine the value of amniotic fluid white blood cell count in the diagnosis of microbial invasion of the amniotic cavity. Amniotic fluid was retrieved by amniocentesis from 195 patients with preterm labor and intact membranes. Fluid was cultured for aerobic and anaerobic bacteria, as well as for mycoplasmas. The prevalence of a positive amniotic fluid culture was 12.8% (25/195). Patients with a positive amniotic fluid culture had a significantly higher median amniotic fluid white blood cell count than did patients with a negative amniotic fluid culture (median, 6 cells/mm3; range, 0 to 11,000 cells/mm³ vs median, 320 cells/mm³; range, 1 to 4480 cells/mm³; ρ < 0.0001). An amniotic fluid white blood cell count ≥50 cells/mm³ had a sensitivity of 80% (20/25), a specificity of 87.64% (149/170), a positive predictive value of 48.78% (20/41), and a negative predictive value of 96.75% (149/154) in the detection of a positive amniotic fluid culture for microorganisms. Although the sensitivity of an amniotic fluid white blood cell count (≥50 cells/mm³) in the detection of microbial invasion of the amniotic cavity was greater than that of the Gram stain of amniotic fluid (80% [20/25] vs 48% [12/25]; p < 0.05), the specificity was lower (87.64% [149/170] vs 98.8% [168/170]; p < 0.05). However, 88% (15/17) of all patients with an amniotic fluid white blood cell count ≥50 cells/mm³ and a negative amniotic fluid culture had a spontaneous preterm delivery. We conclude that the amniotic fluid white blood cell count is a sensitive, simple, and inexpensive test for the detection of microbial invasion of the amniotic cavity. An elevated amniotic fluid white blood cell count is a risk factor for preterm delivery. (AM J OBSTET GYNECOL 1991;165:821-30.)

Key words: Neutrophils, leukocytes, amniotic fluid, preterm labor, prematurity, chorioamnionitis, tocolysis, labor, Gram stain, Mycoplasma, Ureaplasma urealyticum

The rapid and accurate diagnosis of microbial invasion of the amniotic cavity remains an important clinical challenge. Patients with preterm labor and intact membranes with a positive amniotic fluid culture for microorganisms are more likely to have preterm delivery¹⁻⁷ and are at increased risk for both maternal (reviewed in reference 8) and neonatal infection-associated morbidity as compared with patients with a negative amniotic fluid culture.⁸⁻¹⁰

In centers using amniocentesis in the management of preterm labor, the Gram stain is used for the rapid detection of microbial invasion of the amniotic

From the Departments of Obstetrics and Gynecology and Laboratory Medicine, Yale University School of Medicine.

Supported by a grant from the Walter Scott Foundation for Medical Research. Dr. Romero is supported by a Physician Scientist Award from the National Institute of Child Health and Human Development. Presented at the Eleventh Annual Meeting of the Society of Perinatal Obstetricians, San Francisco, California, January 28—February 2, 1991

Reprint requests: Roberto Romero, MD, Department of Obstetrics and Gynecology, 333 Cedar St., PO Box 3333, New Haven, CT 06510. 6/6/31200 cavity.¹⁻¹⁰ However, the Gram stain detects microorganisms in only 50% of cases with a positive amniotic fluid culture.¹¹ Neonatal infection-related morbidity remains high in patients with a false-negative Gram stain¹¹; therefore the search for other methods to identify microbial invasion of the amniotic cavity is justified.

The white blood cell count has been used for the diagnosis of infection in other body fluids such as cerebrospinal fluid, ¹² synovial fluid, ¹³ and pleural fluid. ¹⁴ Previous reports examining the value of white blood cells in amniotic fluid in the diagnosis of microbial invasion of the amniotic cavity have yielded conflicting results. ^{1, 15-19} The purpose of this study was to determine the role of the amniotic fluid white blood cell count in the diagnosis of a positive amniotic fluid culture for microorganisms.

Material and methods

Patient population. One hundred ninety-five consecutive patients admitted to Yale—New Haven Hospital over a 31-month period with the diagnosis of preterm labor and singleton gestation underwent amniocentesis

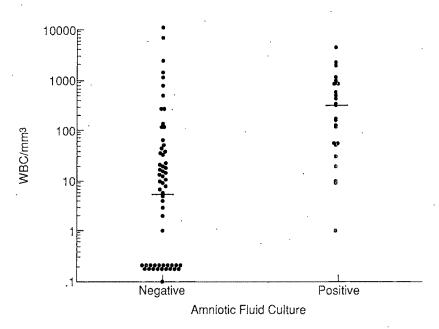


Fig. 1. Amniotic fluid white blood cell counts (WBC/mm³) in patients in preterm labor with intact membranes. Patients with a positive amniotic fluid culture had significantly higher median amniotic fluid white blood cell count than did patients with a negative amniotic fluid culture (median, 320 cells/mm³; range, 1 to 4480 cells/mm³ vs median, 6 cells/mm³; range, 0-11,000 cells/mm³; $\rho = 0.0000001$, Mann-Whitney U test).

for the assessment of the microbiologic status of the amniotic cavity. Preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 minutes. Rupture of membranes was excluded by testing for pooling, Nitrazine paper reaction, and ferning. Patients were hydrated for I hour with at least 1 L of lactated Ringer's solution, and if contractions persisted, the patient was started on a regimen of intravenous tocolysis with ritodrine, terbutaline, or magnesium sulfate. The protocol for tocolysis with β-adrenergic agents followed the guidelines proposed by Caritis.20 Magnesium sulfate was given as a loading dose of 4 gm, followed by a rate of 2 mg/hr, which was increased to 3 to 4 gm/hr if needed. Failure of tocolysis was defined as progressive cervical dilatation beyond 5 cm or delivery of the infant.

Retrieval of amniotic fluid. Amniotic fluid was retrieved by transabdominal amniocentesis, which was performed under antiseptic conditions, with a 22-gauge needle monitored with ultrasound.

Microbiologic culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe. Plating occurred within 30 minutes of collection. Amniotic fluid was cultured for aerobic and anaerobic bacteria, as well as for mycoplasmas, according to methods previously described.²¹

Gram stain examination. Gram stain examination was performed with commercial reagents (crystal violet, saffranin, and Gram's iodine, Difco Laboratories, Detroit) under standard conditions. Stained slides were

examined by trained technologists, and the presence or absence of microorganisms was noted. The results of the Gram stain examinations were communicated to the clinicians. Patients with a negative Gram stain examination underwent tocolysis. Patients with a positive Gram stain examination of amniotic fluid for bacteria were given parenteral antibiotics and were delivered. A positive Gram stain for bacteria was an indication for discontinuing tocolysis.

Amniotic fluid white blood cell count. An aliquot of amniotic fluid was transported to the hematology laboratory and examined under a hemocytometer (Neubauer ruled) for the presence of white blood cells. The absolute white blood cell count was calculated by multiplying the area examined by a factor of 10 per area and expressed as number of cells per cubic millimeter.

Amniotic fluid white blood cell differential count. An aliquot of amniotic fluid was placed in a cytocentrifuge chamber (Cytospin 2, Shandon Scientific, London). The specimen was centrifuged for 10 minutes at 117g, allowed to air-dry, and stained with Wright's stain. The amniotic fluid white blood cell differential was calculated after 100 cells were counted. If the specimen had <100 cells, then 10, 25, or 50 cells were counted, and the differential count was calculated accordingly. The results of either the amniotic fluid white blood cell count or the differential count were not made available to the clinicians.

Criteria for the diagnosis of chorioamnionitis and neonatal sepsis. Microbial invasion of the amniotic cav-

Table I. Clinical characteristics of patients in preterm labor according to amniotic fluid culture results

	Negative amniotic fluid culture $(n = 170)$	Positive amniotic fluid culture (n = 25)	p Value
Maternal age (yr, mean ± SD)	25 ± 4.1	27 ± 4.5	NS 0.001
Gestational age (wk, mean ± SD) Cervical dilatation (cm, median and range)	30 ± 5.2	27.6 ± 3.1	0.001 0.009
oci ilai diamadon (cin, median and range)	(0-6)	(1-8)	0.003
Uterine contractions per 10 min (median and range)	2.3	2.5	NS
	(2-5)	(2-5)	

NS, Not significant.

ity was defined as the presence of a positive amniotic. fluid culture.* Clinical chorioamnionitis was defined according to the criteria proposed by Gibbs.22 Neonatal sepsis was diagnosed by the presence of a positive culture of blood, urine, or cerebrospinal fluid.

Statistical analysis. A Mann-Whitney test was used to compare the amniotic fluid white blood cell count in patients with a positive or negative amniotic fluid culture. Receiver-operator characteristic curve analysis was performed with the True Epistat software package (Epistat Services, Richardson, Tex.). Diagnostic indices (sensitivity, specificity, positive predictive value, and negative predictive value) were calculated for the Gram stain and different cutoff levels of amniotic fluid white blood cell count in the diagnosis of infection. Similarly, diagnostic indices were calculated for different levels of amniotic fluid white blood cell count in the prediction of response to tocolysis. Comparisons between sensitivity and specificity of the Gram stain and amniotic fluid white blood cell counts were performed with a modified t test for correlated samples, as described by Galen and Gambino.23

Results

One hundred ninety-five patients were included in the study; 43% (84/195) had delivered at term. The prevalence of a positive amniotic fluid culture was 12.8% (25/195). Table I describes the clinical characteristics of patients with positive and negative amniotic fluid cultures. Patients with a positive amniotic fluid culture had a significantly lower gestational age and a more advanced cervical dilatation than did patients with a negative amniotic fluid culture. Fig. 1 displays the amniotic fluid white blood cell count according to culture results. Patients with a positive amniotic fluid culture had a significantly higher median amniotic fluid white blood cell count than did patients with a negative amniotic fluid culture (median, 6 cells/mm³; range, 0 to 11,000 cells/mm3 vs median, 320 cells/mm3; range, 1 to 4480 cells/mm³; p < 0.0000001, Mann-Whitney U test).

To describe the relationship between the sensitivity

(true-positive rate) and the false-positive rate (1 – specificity) of amniotic fluid white blood cell count in the detection of a positive amniotic fluid culture, a receiver-operator characteristic curve was constructed. Fig. 2 shows this receiver-operator characteristic curve (area under the curve, 0.823; SE, 0.04; z = 8.110; p < 0.0000001). The curve lies significantly above the 45-degree line, suggesting that a higher amniotic fluid white blood cell count increases the probability of a positive amniotic fluid culture. As with any other diagnostic test, there is a trade-off between the sensitivity and the false-positive rate (1 - specificity).

Table II displays the median percent amniotic fluid white blood cell differential count according to culture results. The median percent of neutrophils and macrophages was significantly higher in patients with a positive amniotic fluid culture than in patients with a negative amniotic fluid culture. Patients with a positive amniotic fluid culture had a significantly higher median absolute amniotic fluid neutrophil count than did patients with a negative amniotic fluid culture (median, 175 cells/mm³; range, 0 to 3853 cells/mm³ vs median, 4 cells/mm³; range, 0 to 10,120 cells/mm³; p = 0.0001, Mann-Whitney U test). Fig. 3 displays the comparison between the receiver-operator characteristic curve of the amniotic fluid white blood cell count and that of the amniotic fluid absolute neutrophil count in the diagnosis of a positive amniotic fluid culture (area under the curve, 0.771; SE, 0.004; z = 6.68; p < 0.05). In the diagnosis of microbial invasion of the amniotic cavity the performance of the amniotic fluid absolute neutrophil count was similar to that of the total amniotic fluid white blood cell count. No difference between the two curves was detected (p = 0.34).

There was no difference in the absolute red bloodcell count between patients with a positive and a negative amniotic fluid culture (median, 168 cells/mm³; range, 8 to 98,000 cells/mm³ vs median, 53; range, 0 to 116,000 cells/mm³, respectively; p > 0.05). There was a correlation between amniotic fluid white blood cell and red blood cell count (r = 0.45, p < 0.05; Spearman's correlation coefficient). However, this correlation

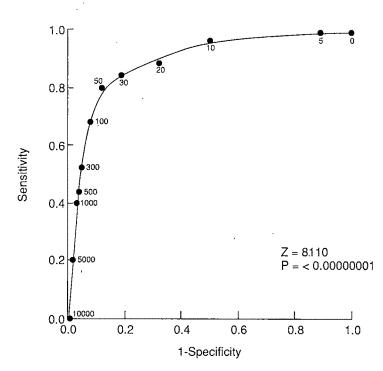


Fig. 2. Receiver-operator characteristic curve analysis of amniotic fluid white blood cell counts in the diagnosis of a positive amniotic fluid culture. *Numbers* next to *solid dots* represent amniotic fluid white blood cell counts (cells per cubic millimeter) (area under curve, 0.823; SE, 0.04; z = 8.110; p < 0.0000001).

Table II. Comparison of amniotic fluid white blood cell differential count between patients with positive and negative amniotic fluid cultures

	Positive culture		Negative	culture	
	Median (%)	Range (%)	Median (%)	Range (%)	p Value
Granulocytes	78	0-100	60	0-100	0.04
Lymphocytes	7	0-30		0-100	NS
Macrophages	14	0-90	3.5	0-100	· 0.003
Eosinophils		0-10	0	0-100	NS

Amniotic fluid white blood cell differential count is expressed as percentage of total white blood cells. NS, Not significant.

is not significant when the analysis is restricted to patients with a positive amniotic fluid culture (r = 0.37, p = 0.07).

Table III displays the diagnostic indices of the Gram stain and the amniotic fluid white blood cell count with different cutoff levels. An amniotic fluid white blood cell count of ≥ 50 cells/mm³ had a greater sensitivity than the Gram stain of amniotic fluid in the detection of a positive culture (80% [20/25] vs 48% [12/25]; p < 0.05).

When the Gram stain and amniotic fluid white blood cell count were used in combination (a positive Gram stain or an amniotic fluid white blood cell count ≥50 cells/mm³ considered abnormal), the diagnostic indices were as follows: sensitivity, 84% (21/25); specificity,

88.82% (151/170); positive predictive value, 52.50% (21/40); negative predictive value, 97.41% (151/155).

One hundred seventy-eight patients had a negative Gram stain and an amniotic fluid white blood cell count of <50 cells/mm³. Four of these patients had a positive amniotic fluid culture (two with *Ureaplasma urealyticum*, one with mixed anaerobic flora, and one with *Candida albicans*). Tocolysis failed in all four patients and all had premature delivery.

Table IV displays the clinical data of the 17 patients with an amniotic fluid white blood cell count ≥50 cells/mm³ and a negative amniotic fluid culture. Eighty-eight percent (15/17) of these patients had refractory preterm labor that led to preterm delivery. Seventy-six percent (10/13) of patients showed histologic evidence

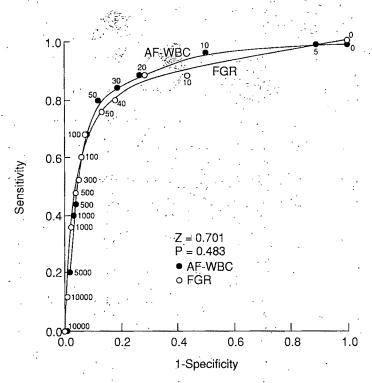


Fig. 3. Comparison of receiver-operator characteristic curve analysis of amniotic fluid white blood cell count (AF-WBC) and absolute granulocyte count (FGR) in the diagnosis of a positive amniotic fluid culture. Numbers next to solid dots represent amniotic fluid white blood cell counts (cells per cubic millimeter) and those next to clear dots represent absolute granulocyte counts (neutrophils per cubic millimeter). No significant difference between these two curves was found.

of chorioamnionitis. One patient was delivered by cesarean section at 35 weeks because of suspected abruptio placentae, which was not confirmed at placental examination. Two patients carried their pregnancies to term; one of them had a total amniotic fluid white blood cell count of 133 cells/mm³ and 116 neutrophils/mm³, and the other had a total amniotic fluid white blood cell count of 130 cells/mm³ and 98 neutrophils/mm³.

Table V displays the clinical course and microbiologic findings of patients with a positive amniotic fluid culture. Twenty-eight percent of patients had polymicrobial infections. Thirteen patients had a negative Gram stain for microorganisms, and nine of these patients had an amniotic fluid white blood cell count >50 cells/mm3.

Clinical chorioamnionitis occurred in 2% (4/195) of patients. Only one patient had a positive amniotic fluid culture (Escherichia coli); the Gram stain of amniotic fluid showed gram-negative rods, and the amniotic fluid white blood cell count was 52 cells/mm3. The placenta showed histologic evidence of chorioamnionitis. The amniotic fluid white blood cell counts of the other patients were 23, 16, and 2 cells/mm3. Only one placenta from these three patients was examined, and no acute inflammatory lesions were found.

No neonate had a positive blood, urine, or cerebrospinal fluid culture. However, two neonates were diagnosed as having presumed sepsis. One was born to a mother who had a positive amniotic fluid culture (Enterococcus) at an outside hospital with a colony count of >10⁵ colony forming units per milliliter and had been treated with ampicillin for a possible urinary tract infection before transfer to our institution. The amniotic fluid culture from repeat amniocentesis performed on admission was negative (Gram stain negative; amniotic fluid white blood cell count, 1 cell/mm³). In view of the early gestational age (26 weeks), it was elected to continue the mother on a regimen of antibiotic therapy in an effort to prolong the pregnancy. Cervical dilatation progressed to 6 cm, and the patient underwent a cesarean section because of breech presentation 1 week later. Intraoperative uterine cultures were positive for Klebsiella pneumoniae and Streptococcus viridans. The first organism was resistant to ampicillin whereas the second demonstrated an intermediate sensitivity to this antibiotic. The placenta showed severe histologic chorioamnionitis. The neonate had multiple complications of prematurity and died after a massive grade IV intraventricular hemorrhage.

The second neonate with presumed sepsis was born to a mother with a positive cervical culture for Streptococcus agalactiae who was unresponsive to tocolysis (amniotic fluid white blood cell count, 22 cells/mm³).

Amniotic fluid white blood cell count Gram stain ≥50 cells/mm³ ≥100 cells/mm³ ≥500 cells/mm³ % % % % No.No.No. No. 12/25 17/25 Sensitivity 48 80 20/25 66 40 10/25 Specificity 98.82 168/170 87.64 149/170 92.94 158/17097.05 165/170 Positive predictive value 20/41 85.71 12/14 48.78 58.62 17/29 66.67 10/15 Negative predictive 92.81 168/181 96.75 149/154 95.18 158/166 91.67 165/180 value

Table III. Comparison of diagnostic indices of Gram stain and amniotic fluid white blood cell count

Table IV. Clinical information and amniotic fluid white blood cell count in patients with false-positive amniotic fluid white blood cell results (≥50 cells/mm³)

Patient No.	Amniotic fluid white blood cell count (cells/mm³)	Gestational age at amniocentesis (wk)	Gestational age at delivery (wk)	Previous antibiotic therapy	Histologic chorioamnionitis
1	2,400	29	33.5	No	No
2	120	30.5	31.5	No	Yes
3	1,380	32.5	32.5	No	Yes
4	11,000	35	35	No	No
5	475	33	33	No	Yes
6	115	24	25	No	N/A
7	6,700	33	33	No	Yes
8	133	31.5	39	No	Yes
9	267	26	26.5	Yes	Yes
10	130	32.5	38.5	No	N/A
11	- 280	·36	36	No	N/A
12	750	35	35	. No	No
13	63	33.6	. 34	No	Yes
14	51	27.5	29	No	N/A
15	66	28.0	28	No	Yes
16	50	26.5	26.5	No	Yes
17	62	. 33	33	No	Yes

N/A, Not available.

The placenta showed eviderice of histopathologic chorioamnionitis. The neonate had a clinical course consistent with sepsis but recovered with antibiotic treatment.

Of the 195 patients included in the study, 156 underwent a trial of tocolysis (12 patients had a positive Gram stain and 37 were delivered for maternal or fetal indications). Fig. 4 displays a receiver-operator characteristic curve for the value of an amniotic fluid white blood cell count in the prediction of preterm labor refractory to tocolysis (area under the curve, 0.625; SE, 0.044; z=2.810; p<0.01). Table VI displays the diagnostic indices of different amniotic fluid white blood cell counts in the identification of the patients who failed to respond to tocolysis. All patients with an amniotic fluid white blood cell count ≥ 500 cells/mm³ had delivery of a preterm neonate.

The amniocentesis-to-delivery interval of patients with a positive amniotic fluid culture for mycoplasmas was significantly shorter than that of patients with sterile amniotic fluid (median, 0.8 days; range, 0 to 23.68

days vs median, 28 days; range, 0 to 122; p < 0.001; Wilcoxon rank sum test).

Comment

Our data show a strong relationship between the amniotic fluid white blood cell count and microbial invasion of the amniotic cavity. This observation can be exploited for diagnostic purposes. In our study an amniotic fluid white blood cell count of ≥50 cells/mm³ had a higher sensitivity than did the Gram stain of amniotic fluid in the detection of a positive culture (80% vs 48%, p < 0.05). The greater sensitivity of the amniotic fluid white blood cell count may be attributed to the inability of the Gram stain to detect mycoplasmas (44% [11/25] of patients with a positive amniotic fluid culture had only mycoplasmas isolated from the amniotic fluid). Indeed, only two of the 11 patients in whom mycoplasmas were the only amniotic fluid isolates had a positive Gram stain. Since mycoplasmas are not seen with a Gram stain examination, these cases represent either "false-positive" Gram stains or failure

Table V. Amniotic fluid white blood cell count, microbiologic studies, and placental histopathologic findings in patients with positive amniotic fluid culture

Patient . No.	Amniotic fluid white blood cell count (cells/mm³)	Total granulocyte (cells/mm³)	Gram stain	Organism	Colony count (cfu/ml)	Gestational age at amniocentesis (wk)	Gestational age at delivery (wk)	Histologic chorioamnionitis
1	330	303		Fusobacterium sp.	_	25.5	25.5	+
2	30	21		Ureaplasma urealyticum	_	31	31	+
3	10	14	_	Ureaplasma urealyticum	_	30	30.5	+
4	162	39	+	Bacteroides fragilis	$>10^{5}$	26	26	+
				Fusobacterium sp.	1+			
. 5	54	50	_	Ureaplasma urealyticum	_	32	32	_
6	1000	600	+	Bacteroides fragilis	>105	. 24	25	+
				Enterococcus fecalis	1+			
7	.52	33	+	Mycoplasma hominis	- ·	24.5	24.5	+
8	580	510	-	Ureaplasma urealyticum	_	31	31	+
9	502	397	_	Ureaplasma urealyticum	_	24.5	24.5	+
10	130	117	+	Mixed anaerobic or- ganisms	17,000	26	26	+
11	430	396	+	Mycoplasma hominis	$>10^{5}$	27	30	_
12	1	0	_	Mixed anaerobic organisms	- .	30.9	31	+
13	173	118	_	Ureaplasma urealyticum	_	24.6	24.7	+
14	4480	3853	+	Peptostreptococcus sp.	$> 10^{5}$	33.9	34	+
15	835	660	+	Fusobacterium sp.	$> 10^{5}$	27.9	28	+
				Mycoplasma hominis				
16	810	648	+	Gardnerella vaginalis	$>10^{5}$	27.9	28	+
17	320	230	_	Mycoplasma hominis	1+	23	24	+
18	2150	1763	_	Staphylococcus aureus	4 +	32.9	33	+
19	1100	748	+	Fusobacterium sp.	$> 10^{5}$	30.9	31	+
20	19	18	+	Streptococcus agalactiae	$> 10^{5}$	25.4	25	N/A
21	56	0	+	Capnocytophaga Clostridium sp.	4+ 4+	25.9	26	+
22	9	. 0	_	Candida albicans	_	24.4	26	+
23	1970	1793	+	Gardnerella vaginalis	$>10^{5}$	23.9	24	+
***				Peptostreptococcus sp.	1+	23.9	24	
24	114	80		Ureaplasma urealyticum	_	28.8	29	+
25 .	1100	. 660		Ureaplasma urealyticum		27.9	28	+

N/A, Not available.

to recover fastidious microorganisms in cases of mixed infections. On the other hand, an amniotic fluid white blood cell count ≥ 50 cells/mm³ was present in 81.8% (9/11) of patients in whom mycoplasmas were the only

The greater sensitivity of the amniotic fluid white blood cell count in the detection of a positive culture of amniotic fluid in comparison with the Gram stain was associated with a lower specificity (87.6% vs 98.8%, respectively; p < 0.05). The resulting higher falsepositive rate may be an important shortcoming of the amniotic fluid white blood cell count. However, the apparent false-positive results deserve comment. Eightyeight percent (15/17) of patients with an amniotic fluid white blood cell count ≥ 50 cells/mm³ and a negative amniotic fluid culture had premature delivery, and 66% (10/13) of them showed histologic evidence of chorioamnionitis. Therefore, independent of the culture result, an elevated amniotic fluid white blood cell count identified a subset of patients at risk for failure to respond to tocolysis and impending preterm delivery. These patients may have had microbial invasion of the amniotic cavity that escaped detection with the microbiologic surveillance techniques used in our study or, alternatively, neutrophil recruitment into the amniotic cavity may have been driven by a noninfectious process.

Five patients had false-negative amniotic fluid white blood cell counts (<50 cells/mm³) and a positive amniotic fluid culture. Three potential explanations may be invoked for these findings: (1) failure of the host to respond to microbial invasion of the amniotic cavity, (2) low virulence of microorganisms involved, or (3) a time-dependent phenomenon. If microbial invasion of the amniotic cavity is of short duration (i.e., it occurred after the onset of preterm labor), the host may not have had enough time to mount an inflammatory response. Regrettably, the relative contribution of these mechanisms cannot be addressed without data.

The white blood cell differential count has diagnostic value in peripheral blood and cerebrospinal fluid. Does it have value in amniotic fluid? Patients with a positive amniotic fluid culture had a significantly higher abso-

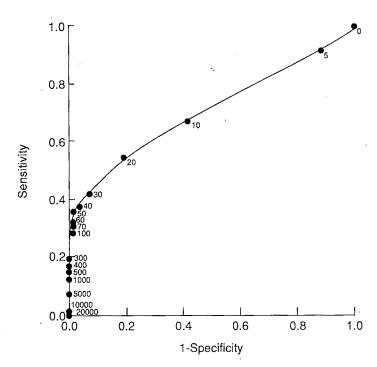


Fig. 4. Receiver-operator characteristic curve analysis of amniotic fluid white blood cell count in identification of patient who will have delivery of preterm neonate. Only patients with negative Gram stain of amniotic fluid who received tocolysis were included in this analysis. *Numbers* next to *solid dots* represent amniotic fluid white blood cell counts (cells per cubic millimeter) (area under curve, 0.625; SE, 0.044; z = 2.810; p < 0.01).

Table VI. Diagnostic indices of amniotic fluid white blood cell count in prediction of spontaneous preterm delivery

Amniotic fluid white blood cell count (cells/mm³)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
≥500	12.5 (9/72)	100 (84/84)	100 (9/9)	57.1 (84/147)
≥100	27.7 (20/72)	98.8 (83/84)	95.2 (20/21)	61.4 (83/135)
≥50	31.9 (23/72)	98.8 (83/84)	95.8 (23/24)	62.8 (83/132)

lute neutrophil count than did patients with a negative amniotic fluid culture. However, we could not demonstrate any difference between the receiver-operator characteristic curves of the absolute neutrophil count and the total amniotic fluid white blood cell count (Fig. 3). The most likely explanation for this observation is that in preterm labor the neutrophil is the white blood cell most commonly recruited into the amniotic cavity (see Table II). The practical consequence of this observation is that a simple amniotic fluid white blood cell count has diagnostic value comparable to that of the absolute neutrophil count, which requires a centrifugation step, staining, and reading of the smear. However, these data do not negate the potential value of the amniotic fluid white blood cell differential. Further studies are required to address the frequency and value of other white blood cell types in the diagnosis of other intrauterine pathologic conditions (e.g., viral disease, immune-mediated phenomena, etc.).

An elevated amniotic fluid white blood cell count was not associated with clinical chorioamnionitis. This suggests that the inflammatory response was localized to the uterine cavity and did not elicit a clinically demonstrable systemic response (e.g., fever). Indeed, preterm parturition may be considered as an efficient mechanism to limit the dissemination of intrauterine infection and to prevent systemic maternal disease. We were surprised to find a low amniotic fluid white blood cell count in three of the four patients with clinical chorioamnionitis. These cases may represent extraamniotic infections (e.g., deciduitis) or clinical diagnostic errors.

The absence of proved neonatal sepsis in our study must be interpreted with caution since patients with a positive Gram stain for microorganisms were treated antepartum with antibiotics, and this may have altered neonatal culture results. Moreover, neonatal cultures for mycoplasmas, the most common microbial isolates in our study, were not performed. It is possible that a fraction of the suspected but unproved neonatal sepsis may be due to these organisms.24.25

An important additional observation of this study is that patients with microbial invasion of the amniotic cavity as a result of mycoplasmas had a significantly shorter amniocentesis-to-delivery interval than did patients with a negative amniotic fluid culture. Moreover, 81.8% (9/11) of patients with a positive amniotic fluid culture and genital mycoplasmas had an amniotic fluid white blood cell count ≥50 cells/mm³ and also had histologic evidence of chorioamnionitis (Table V). These observations indicate that microbial invasion of the amniotic cavity with mycoplasmas is associated with recruitment of white blood cells into the amniotic cavity and also with acute inflammatory lesions of the placenta. These findings, coupled with the isolation of Ureaplasma urealyticum and Mycoplasma hominis from the cerebrospinal fluid24 and bronchial secretions25 of preterm neonates who had meningitis and chronic lung disease, support a pathogenic role of these microorganisms. However, our results are at variance with those of Gravett et al.,6 who found no difference in amniocentesis-to-delivery interval between patients with sterile amniotic fluid and those with a positive amniotic fluid culture for genital mycoplasmas. Moreover, they found virtually no amniotic fluid white blood cells in patients with positive amniotic fluid cultures for genital mycoplasmas. We have no explanation for the discrepancy between the two reports.

We have examined the combined use of the amniotic fluid white blood cell count and Gram stain examination of amniotic fluid in the rapid diagnosis of microbial invasion of the amniotic cavity. Simultaneous use of both tests was associated with an increased sensitivity in comparison to that of the Gram stain alone (84% vs 48%, p < 0.05). However, this was associated with an increased false-positive rate (1.2% to 11.2%; p < 0.05). We believe this false-positive rate is more apparent than real because 88% of patients with an elevated amniotic fluid white blood cell count and a negative culture had a spontaneous preterm delivery. These findings call for caution in the use of the amniotic fluid culture as the gold standard in this type of study. A positive amniotic fluid culture requires a minimum inoculum size, viability of the microorganisms in the specimen, and successful recovery of the microorganisms in the microbiology laboratory. Moreover, microorganisms unidentified by current laboratory techniques also may be responsible for some intrauterine infections. Our data indicate that an elevated amniotic fluid white blood cell count identifies patients with microbial invasion of the amniotic cavity and a population of patients at risk for preterm delivery despite a negative amniotic fluid culture. It remains to be established if the inflammatory reaction in these patients is caused by an unidentified infectious agent or a non-infection-driven

Simultaneous use of the Gram stain and the amniotic fluid white blood cell count takes advantage of the high specificity of the Gram stain for the detection of microbial invasion of the amniotic cavity and of the high specificity of amniotic fluid white blood cell count for the prediction of preterm delivery. The patient with a negative Gram stain of amniotic fluid and a white blood cell count <50 cells/mm3 had only a 2.8% risk of having a positive culture and was at low risk for having a preterm birth (3.7%). On the other hand, a patient with a positive Gram stain of amniotic fluid and an elevated amniotic fluid white blood cell count has nearly a 100% risk of having a positive amniotic fluid culture (100% [11/11] had a positive amniotic fluid culture). In our opinion, this is an indication for antibiotic treatment and for withholding tocolytic therapy. We were unable to estimate the risk of spontaneous preterm delivery for these patients because the Gram stain was used for intervention in some patients in our study. A more difficult management problem is the patient with a negative Gram stain of amniotic fluid and a white blood cell count above 50 cells/mm3. These patients are likely to have microbial invasion of the amniotic cavity with Mycoplasmas or an extraamniotic infection (deciduitis) or an inflammatory process unrelated to infection. The development of rapid methods for the diagnosis of Mycoplasma infections can contribute to the identification of the first condition. Further studies are required to develop methods to detect deciduitis of infectious or noninfectious etiology.

We conclude that the amniotic fluid white blood cell count is a rapid, sensitive, inexpensive, and simple test for the detection of microbial invasion of the amniotic cavity and for the identification of patients at risk for spontaneous preterm delivery.

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Shoulder dystocia: Should the fetus weighing ≥4000 grams be delivered by cesarean section?

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A total of 75,979 women who were delivered vaginally in the period 1970 to 1985 were stratified into diabetic and nondiabetic groups. Overall, the incidence of macrosomia (≥4000 gm) was 7.6% (5674/74390) in the nondiabetic group and 20.6% (328/1589) in the diabetic group. Patients were further subdivided by weight categories at 250 gm intervals. Eight percent of shoulder dystocia occurred in the diabetic group when fetal weight was ≥4250 gm. In contrast, 20% of shoulder dystocia in the nondiabetic group could have been prevented by elective cesarean section when the fetal weight was ≥4500 gm. Furthermore, logistic regression analysis demonstrated that birth weight, diabetes, and labor abnormalities were the principal contributors to shoulder dystocia. Elective cesarean section is strongly recommended for diabetics with fetal weights ≥4250 gm, and trial of vaginal delivery for nondiabetic fetuses with weights ≥4000 gm is recommended. In all cases the clinician must be watchful for labor abnormalities in macrosomic fetuses. (AM J OBSTET GYNECOL 1991;165:831-7.)

Key words: Shoulder dystocia, diabetes in pregnancy, fetal macrosomia, trauma

Shoulder dystocia is the infrequent, unanticipated, unpredictable nightmare of the obstetrician. The overall reported incidence of shoulder dystocia is 2 to 21 per 1000 births. The care provider may have limited skills to manage this catastrophe because of its infrequency.

Perinatal outcome associated with shoulder dystocia results in increased morbidity and mortality. Additionally, this clinical calamity often may be associated with disastrous medical-legal results. Therefore most authorities recommend that an elective cesarean section be performed when estimated fetal weight is ≥4500 gm. However, controversy persists on the optimum method of delivery of the fetus weighing 4000 to 4500 gm.²⁻⁴

The frequency of shoulder dystocia is believed to be higher in diabetic subjects. In spite of this fact, the majority of studies addressing shoulder dystocia failed to stratify the patient populations into diabetic and non-diabetic groups. This is a crucial omission because diabetes mellitus is an accepted risk factor for macrosomia, shoulder dystocia, and birth trauma. The rarity of shoulder dystocia has predisposed studies to smaller sample sizes that are insufficient to demonstrate the

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true prevalence of shoulder dystocia within multiple fetal weight categories (Table I). Thus there have been limitations on the in-depth analysis of the magnitude of the problem, which may have affected the results reported in the studies.

Although several studies have examined the risk factors for shoulder dystocia, paucity of information exists regarding the net effect of these risk factors on diabetic and nondiabetic women. Our research addressed the question: Is cesarean section the optimum method of delivery of the fetus ≥4000 gm? The measurable objectives were to ascertain the effects and relative contribution of risk factors (by means of a logistic regression model) for diabetic and nondiabetic subjects. This would provide an estimate of the net effect of each risk factor and identify the potential high-risk pregnancies that should be monitored with greater intensity.

Material and methods

A trend study of 75,979 consecutive vaginal deliveries (fetal weight, ≥2500 gm) from 1970 to 1985 was undertaken by the Department of Obstetrics and Gynecology at The University of Texas Health Center at San Antonio. Data, consisting of medical history, demographic characteristics, obstetric complications, labor and delivery events, and neonatal outcome, were available from the computerized departmental data base.

Shoulder dystocia was defined as failure of the shoulder to be delivered after the head in spite of standard maneuvers that were based on the judgment of the clinician delivering the fetus. Diabetes mellitus included

	Shoulder dystocia		Incidence		Diabetics	Macrosomia in
Name	Year	(No.)	Percent	No.	segregated	study population (%)
Benedetti et al:4	1978	14	0.2	8,474	No	7.8
Johnstone ⁶	1979	47	0.2	22,663	No	7.0
Hopwood ⁸	1982	92	0.5	17,735	No	` NA
Acker et al.2	1985	309	2.1	14,721	Yes	9.0
					(n = 144)	•
Gross et al.⁵	1987	24	0.2	10,662	No	NA
Gross et al.3	1987	116	1.7	7,123	No	5.5
Sandmire and Halloin!	1987	73	0.5	14,806	No	11.9
Hassan ⁷	1988	41	0.2	18,889	No	NA
Current study	1991	456	0.6	75,979	Yes	7.7
•					(m - 1580)	

Table I. Incidence of shoulder dystocia and associated fetal macrosomia in selected articles

NA. Not available.

pregestational and gestational individuals. Outcome measures included (1) trauma (nerve injury, fracture, and seizures), (2) pulmonary complications (meconium aspiration, pneumothorax, and neonate requiring ventilation support), (3) Apgar score < 7 at 5 minutes with division into two categories, 1 to 3 and 4 to 6, and (4) perinatal mortality.

For purpose of analysis, patients were classified into diabetic and nondiabetic groups. Patients were further stratified into birth weight categories at 250 gm intervals beginning at 3750 gm. The birth weight category between 2500 and 3749 gm served as the normal-weight reference group against which other weight categories were compared. Comparison within and between groups was performed on all categoric data by means of χ^2 and Fisher's exact tests where appropriate.

To assess the extent to which various risk factors affect shoulder dystocia, we used the logistic regression model with the BMDP statistical package (BMDP Statistical Software, 1988, Los Angeles). The resulting odds ratios indicated the net effect of a given independent variable when all other variables were controlled.

Results

Patient characteristics. During this study period 74,390 nondiabetic and 1589 diabetic patients were delivered. Overall the incidence of macrosomia (≥4000 gm) in the nondiabetic group was 7.6% (5674/74390), whereas an incidence of 20.6% (328/1589) was found in the diabetic group.

The race distribution was comparable for diabetic and nondiabetic patients; approximately 10% white, 10% black, and 80% Hispanic. Diabetic subjects were significantly older than the nondiabetic ones (28 \pm 6.5 vs 23 \pm 5.5, p < 0.0001). Additionally, more diabetic subjects were >30 years old in comparison with nondiabetic women (44.0% vs 12.7%, p < 0.0001). Multiparity accounted for 78.9% of the diabetic versus 58% for nondiabetic women (p < 0.0001).

The overall incidence of shoulder dystocia was 3.1% (range, 1.8% to 4.8%) for diabetic women and 0.5% (range, 0.4% to 0.7%) for nondiabetic women. The relative risk for shoulder dystocia was significantly higher for diabetic versus nondiabetic women (relative risk, 5.92; 95% confidence interval, 4.39 to 7.98). Further analysis revealed that the relative risk for shoulder dystocia was 2.6-fold (95% confidence interval, 1.29 to 5.34) higher for diabetic versus nondiabetic women in the weight category <4000 gm and 3.6-fold (95% confidence interval, 2.37 to 4.76) higher for diabetics when fetal weight was ≥4000 gm.

Association between fetal weight and shoulder dystocia. Overall, there was a threefold higher frequency of macrosomic infants born to diabetic than to nondiabetic mothers. Table II demonstrates the frequency of large infants within each birth weight category. As anticipated, when cases were grouped in 250 gm increments, there was an increased incidence of shoulder dystocia directly related to increased infant birth weight. With 4500 gm used as a threshold, there was a twofold higher rate of macrosomic infants in the diabetic group (33%) when compared with the nondiabetic group (16%).

The relationship between shoulder dystocia and birth weight categories revealed that approximately 40% of shoulder dystocia in the nondiabetic group occurred in infants weighing <4000 gm. In the diabetic group 84% showed evidence of shoulder dystocia with birth weights ≥4000 gm (Fig. 1).

Finally, when the incidence of shoulder dystocia was evaluated by birth percentile to control for the effect of gestational age on birth weight, it was found that 87% of the cases occurred in the ≥90th birth weight percentile for the nondiabetic and in 77% for the diabetic group. Moreover, diabetic infants had a significantly higher risk for shoulder dystocia at the 75th percentile when compared with nondiabetic infants (Fig. 2).

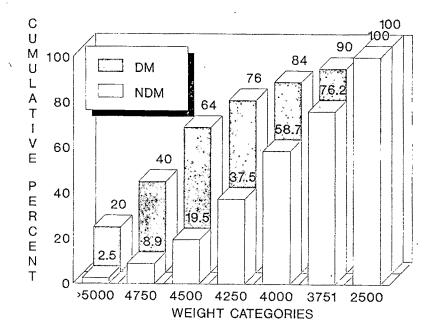


Fig. 1. Percent cumulative incidence of shoulder dystocia at or above stated birth weight for diabetic (DM) and nondiabetic (NDM) pregnant women. For each birth weight threshold ≥3751 gm, cumulative incidence of shoulder dystocia was significantly higher in diabetic subjects.

Table II. Shoulder dystocia as related to birth weight in diabetic and nondiabetic pregnant women

Weight category (gm)	•	Diabetic					
	No.	Shoulder dystocia	%	No.	Shoulder dystocia	%	p Value
2500-3749	1,002	5	0.5	61,569	97	0.2	0.0000
3750-3999	251	3	1.2	6,979	71	1.0	NS
4000-4249	128	4	3.0	3,231	86	2.6	NS
4250-4499	81	6	6.9	1,399	73	5.0	0.03
4500-4749	43	12	21.8	528	43	7.5	0.0000
4750-4999	18	10	35.7	176	26	12.9	0.0000
≥5000	16	10	38.5	102	10	8.9	0.0000
TOTAL	1,539	50		73,984	406		

NS, Not significant.

Relationship between shoulder dystocia and pregnancy outcome. The overall relative risk for pregnancy complications (perinatal mortality, infant trauma, and low Apgar score) was 10.49-fold higher (95% confidence interval, 9.51 to 12.97) in infants with shoulder dystocia when compared with infants without shoulder dystocia. The relative risk for trauma complications was comparable for the diabetic and nondiabetic infants with shoulder dystocia. The diabetic infant had a higher relative risk for perinatal mortality and low Apgar scores when compared with the nondiabetic infant (Table III).

In the subjects with shoulder dystocia, 37% in the nondiabetic and 81% in the diabetic groups showed evidence of at least one or more complications. Among the infants with complications, comparison between the

diabetic and nondiabetic groups revealed the following: (1) that more than twice as many stillbirths occurred in the diabetic group as in the nondiabetic group (25% vs 10%), (2) that there was a comparable rate of neonatal death for both groups (5% and 4%, respectively), (3) that an Apgar score of <7 at 5 minutes showed comparable rates (47% vs 43%), and (4) that in regard to neonatal pulmonary complications similar results were obtained for both groups (7% and 10%, respectively).

The cumulative incidence for shoulder dystocia was 76%, and shoulder dystocia-related trauma was 82% for diabetic infants in the 4250 gm weight group category, with only a minimal 6% increase in the cumulative rate for shoulder dystocia at the 4000 gm category (Fig. 3, A). In contrast, in the nondiabetic group, in the 4500 gm weight category only 20% of shoulder dystocia

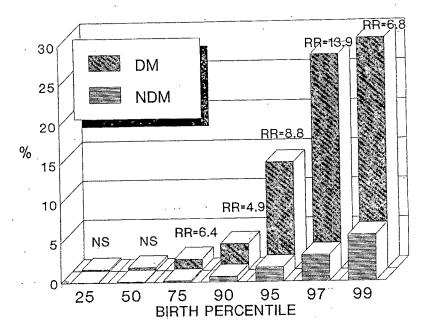


Fig. 2. Frequency of shoulder dystocia and relative risk (*RR*) in each birth weight percentile category for diabetic (*DM*) and nondiabetic (*NDM*) subjects. *NS*, Not significant.

Table III. Association between adverse outcome of pregnancy and shoulder dystocia in diabetic and nondiabetic pregnant women

	Di	abetic			Non	adiabetic		
	With shoulder dystocia (n = 50) (%)	Without shoulder dystocia (n = 1539) (%)	Odds ratio	95% confidence interval	With shoulder dystocia (n = 406) (%)	Without shoulder dystocia (n = 73,984) (%)	Odds ratio	95% Confidence interval
Perinatal mortality Birth trauma Low Apgar score	28 36 38	2.2 3.5 5.8	17.21 15.47 11.06	8.51-34.83 8.17-29.28 5.90-20.44	5.2 16.3 16.3	0.6 1.5 2.9	9.53 14.05 6.40	6.98-14.94 10.72-18.43 4.92-8.39

and shoulder trauma cases were identified. Furthermore, even at 4000 gm almost 40% will go undetected (Fig. 3, B).

Finally, we compared the association between neonatal complications and shoulder dystocia and neonatal complications and shoulder dystocia-associated trauma. In general, complications showed a greater association with shoulder dystocia alone than with shoulder dystocia-associated trauma (p < 0.0001). The comparison between shoulder dystocia and shoulder dystocia and trauma revealed: (1) For pulmonary complications there was an overwhelming 86% versus 14%, respectively; (2) for Apgar scores of 1 to 3 the shoulder dystocia group showed 40% in comparison with 2% for shoulder dystocia-associated trauma with comparable rates, 48% and 9%, respectively, for Apgar scores of 4 to 6; (3) stillbirth occurred only in the shoulder dystocia group; (4) neonatal death in the shoulder dystocia group accounted for 87% and 13% in the shoulder dystocia-associated trauma group.

Identified and rank risk factors contributing to shoulder dystocia. Logistic regression analysis was used to study the effects of each factor on shoulder dystocia while the effects of all other factors were being simultaneously studied. Three analyses were performed: (1) for all the study population, (2) diabetic alone, and (3) nondiabetic alone. The results of the analyses demonstrating the net effect of each factor are summarized in Table IV. Strong associations were found for three of the 12 variables tested for the diabetic group and five of the 12 for the nondiabetic group. In general, birth weight and diabetes showed the highest ranking for increased risk for shoulder dystocia.

Shoulder dystocia and method of delivery. Table V demonstrates the theoretic net cumulative contribution to the overall cesarean section rate if all patients from that weight threshold, or greater, underwent cesarean section. For example, if all diabetic subjects whose infants weigh ≥4250 gm were delivered by cesarean section, the overall cesarean section rate would increase

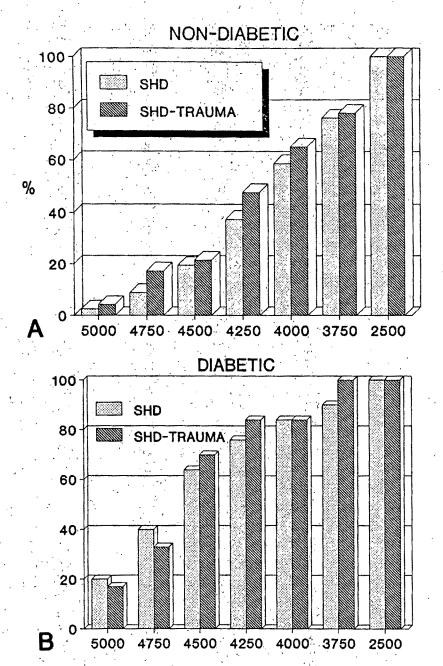


Fig. 3. A, Cumulative incidence of shoulder dystocia (SHD) alone and shoulder dystocia-trauma in each weight category for nondiabetics. B, Cumulative incidence of shoulder dystocia (SHD) alone and shoulder dystocia-trauma in each weight category for diabetics.

by only 0.26% and would result in the elimination of 76% of the cases of shoulder dystocia.

Comment

The key finding in our study is that in diabetic women approximately 80% of the cases of shoulder dystocia with and without trauma can be eliminated by cesarean section delivery, with negligible increase in the overall cesarean section rate. The current cesarean section rate in our institution is 12.5%. Using 4250 gm as the birth weight threshold for elective cesarean section delivery

in our diabetic patients will commit 158 patients to cesarean section. Of these, 24% are associated with shoulder dystocia. However, this 24% represents 76% of the total number of cases in the diabetic group. Thus, if our ultimate goal is the elimination of shoulder dystocia, prevention of 76% for the price of a fractional percentage increase in cesarean section rate appears to be justified. Notably, this represents an insignificant increase in the overall cesarean section rate of, at most, 0.26% (i.e., 12.76% cesarean section rate).

In contrast, in the nondiabetic group no definitive

Table IV. Net contribution of variables affecting shoulder dystocia: Logistic regression analysis

		Odds ratio		
	Diabetic $(n = 50/1539)$	Nondiabetic (n = 406/73,984)	All (N = 456/75,979)	Reference
Diabetes		_	6.0	No diabetes
Parity	NS	NS	NS	Primigravid
Age	NS	NS	NS	<20
Race				
Black	NS	1.2	1.2	White
Sex	NS	NS	NS	Female
Postdatism	NS	NS	NS	Term
Duration of labor	1.3	2.1	1.9	<3 hr
Abnormal labor	NS	NS	NS	Normal
Augmentation	NS	NS	NS	Normal .
Anesthesia				
Paracervical	NS	6.5	6.7	None
Epidural	NS	1.9	2.2	None
Type of delivery				Spontaneous
Vacuum	1.4	2.9	2.5	•
Midforceps	1.7	3.6	3.0	
Birth weight (gm)				2500-3750 gm
3750-3999	6.9	2.2	6.6	O
4000-4249	18.9	5.7	17.6	
4250-4499	. 34.9	13.1	33.1	
4500-4749	78.0	66.7	79.4	
4750-4999	263.0	156.7	236.8	
≥5000	275.6	185.0	305.7	

Table V. Net cumulative contribution to overall cesarean section by weight categories

	Di	abetic	Nondiabetic		
Weight (gm)	Net increase in cesarean section (%)	Shoulder dystocia (%)	Net increase in cesarean section (%)	Shoulder dystocia (%)	
2500-3750	0.76	90	16.8	76.0	
4000	0.43	84	7.5	58.7	
4250	0.26	76	3.1	37.5	
4500	0.14	64	1.2	19.5	
4750	0.07	40	0.4	8.9	
≥5000	0.03	20	0.1	2.5	

weight category was identified as the optimal threshold for cesarean section delivery to decrease cases of shoulder dystocia. Even at 4000 gm, approximately half the cases will go undiagnosed and will be accompanied by an alarming increase in overall cesarean section rate of 7.5%. Moreover, the current recommended weight category of 4500 gm for elective delivery by cesarean section in nondiabetic women is questionable in light of our data. Only 20% of the shoulder dystocia cases will be prevented with the addition of 1.2% to the overall cesarean section rate. Thus the majority of cases of shoulder dystocia (80%) still remain the Pandora's box of the clinician.

Previous studies used bivariate analysis to identify risk factors contributing to shoulder dystocia. 1, 2, 4, 9 Only one study³ attempted to create a predictive model with

three-way discriminant analysis, and it was found that even the best classification failed to disclose 85% of cases. In this study we used the multivariant stepwise logistic regression model, which assigns the relative net importance of each risk factor while all other factors are kept constant. For example, a relative risk of 1.23 for the risk factor race can be interpreted to mean that when adjustment is made for certain other risk factors, black fetuses run a roughly 23% increased risk for shoulder dystocia. It was found that birth weight and the presence of diabetes were the highest risk factors for shoulder dystocia, followed by labor-related factors such as duration of labor, type of anesthesia, and type of delivery. These findings corroborate previous studies.2,4 Other variables previously designated as relevant by bivariate testing, such as parity, maternal age, fetal gender, and postdate pregnancy, failed to retain a significant relationship to shoulder dystocia in the presence of associated factors.3,7

A high complication rate of 37% was found for the nondiabetic group, and an inordinately high rate of 81% was found for the diabetic group. On comparison of each group with its relevant reference group, an increased risk ranging from 6.4 to 15.5 was found. The high incidence of complications for the nondiabetic subjects is consistent with previous reports.3,5 Shoulder dystocia is a clinical diagnosis that is based on the judgment of the clinician and, regardless of accompanying trauma, shoulder dystocia is the culprit. In our study most of the perinatal mortality and morbidity were associated with cases of shoulder dystocia without trauma. Thus shoulder dystocia should be addressed as a formidable obstetric complication, and the accessory complications should be an integral part of the diagnosis. This finding was found to be true for both diabetic and nondiabetic groups.

Our study substantiates the findings of previous studies that demonstrated that approximately 50% of the infants with shoulder dystocia weighed <4000 gm.10 Our study, which stratified subjects into diabetic and nondiabetic groups, demonstrated that the 40% shoulder dystocia rate in the nondiabetic group occurred in infants weighing <4000 gm. Approximately 87% of these infants were relatively large for their gestational age and were classified as ≥90th percentile. Our study disclosed that, in contrast to the nondiabetic subjects, only 20% of shoulder dystocia in diabetic subjects occurred in infants weighing <4000 gm. However, shoulder dystocia was a significant event for infants even at the 75th percentile. Furthermore, a sixfold higher risk for shoulder dystocia occurred in diabetic compared with the nondiabetic women at this percentile classification. Researchers performing anthropometric studies post partum on macrosomic infants described them as having a greater shoulder-head and chest-head disproportion, regardless of birth weight.11 Others, using ultrasonography on antepartum macrosomic infants, recommended the use of a macrosomic index to detect shoulder dystocia.12

In summary, shoulder dystocia and the high perinatal mortality and morbidity associated with this condition necessitate a preventive strategy. For diabetic women elective cesarean section is strongly recommended when estimated fetal weight is ≥4250 gm. This approach will eliminate 80% of cases with a minimal increase in the cesarean section rate. Unfortunately, this is not the case for nondiabetic subjects, because prevention of shoulder dystocia by elective cesarean section will leave the majority of cases undetected. Thus trial of vaginal delivery for nondiabetic fetuses with weight estimation ≥4000 gm is recommended. In these cases the clinician must heed labor abnormality indicators in the presence of the macrosomic infant.

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A five-year statewide experience with congenital diaphragmatic hernia

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Antenatal in utero surgery for congenital diaphragmatic hernia has been justified by reported perinatal mortality rates of 77% to 80%. Such rates may have been subject to bias of ascertainment and may include fetuses with additional severe malformations who would not be surgical candidates. We used the lowa Birth Defects Registry to conduct a complete population survey to determine the incidence of congenital diaphragmatic hernia, the frequency of associated severe malformations, and the morbidity and mortality of infants with isolated congenital diaphragmatic hernia who were not subjected to antenatal surgery. The incidence of congenital diaphragmatic hernia was 1 in 3715. Twenty-eight percent of affected fetuses had associated severe malformations that were potentially identifiable prenatally and that would have precluded antenatal surgery. Of those with isolated congenital diaphragmatic hernia, 55% survived in spite of delivery (88.5%) and/or surgical repair (44%) in a level I or II hospital. Any decision for in utero surgery to repair congenital diaphragmatic hernia must be based on this or similarly obtained information. (AM J OBSTET GYNECOL 1991;165:838-42.)

Key words: Congenital diaphragmatic hernia, in utero surgery

As techniques of fetal diagnosis and treatment have evolved, considerable attention has focused on the possibility of antenatal surgical therapy for select malformations. One potential malformation amenable to prenatal surgical correction is congenital diaphragmatic hernia.1.2 This surgery entails risks to both mother and child, with a reported fetal mortality rate of 75% (6/8) early in the development of this procedure and with associated maternal morbidity that includes chorioamnionitis, preterm labor and delivery, and the creation of an unstable uterine scar dictating repeat cesarean sections for future pregnancies.1.2 This still-experimental surgery has been justified by the reportedly high mortality rate (77% to 80%) of fetuses with congenital diaphragmatic hernia who are not treated until after birth. These rates were derived from physician surveys,3 the experience of single tertiary care centers,4-6 and infant death registries.7 Such data, however, may be biased by referral patterns3-6 and incomplete ascertainment.7 Before fetal surgery for congenital diaphragmatic hernia can be considered an appropriate

option, the natural history of this malformation must be determined as accurately as possible. We used the Iowa Birth Defects Registry to conduct a complete population survey from which we obtained information concerning the incidence and associated morbidity and mortality of congenital diaphragmatic hernia among fetuses delivered with this defect.

Material and methods

All fetuses with congenital diaphragmatic hernia who were born to Iowa residents between 1983 and 1988 were identified through the Iowa Birth Defects Registry. The staff of this registry, which is a cooperative effort involving the University of Iowa, the Iowa Department of Public Health, and the Centers for Disease Control, United State Public Health Service, confidentially investigates all infants with congenital malformations who are born to Iowa residents at >20 weeks' gestation or who weigh >500 gm. Fetuses or infants with birth defects recorded on birth certificates and/or death certificates are identified, and all hospital records (prenatal, labor and delivery, neonatal, and subsequent hospitalizations) are thoroughly reviewed on site by specially trained field representatives. Only fetuses or infants with confirmed diagnoses of congenital diaphragmatic hernia (Bochdalek or Morgagni hernias or congenital absence of the diaphragm) were included in this study.

Information obtained from each case study included maternal history, family history, prenatal course, labor and delivery events, level of the delivery hospital, level of the hospital that provided postnatal care, diagnostic

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Table I. Outcome of infants with congenital diaphragmatic hernia and additional severe malformations

Malformation	No.	. Outcome
Trisomy 18	3	Stillbirths at 32.5 and 33 wk; death on 1st day of life
Trisomy 13	1	Stillbirth at 36.5 wk
Neural tube defect	3	Stillbirths at 28.5, 32, and 35.5 wk
Hypoplastic left heart	2	Stillbirths at 36 and 40 wk
Hypoplastic right heart	1	Stillbirth at 31.5 wk
Multiple malformations	1	Stillbirth at 35.5 wk
Cornelia de Lange syndrome	2	Death on 1st day of life
Pentalogy of Cantrell	1	Stillbirth at 28 wk
Aortic malformation	1	Death on 1st day of life
Cystic hygroma, hydrops	1	Stillbirth at 22.5 wk
Potter syndrome	1	Stillbirth at 39 wk
Microcephaly	I	Alive at 28 mo (seizure disorder, severe developmental delay)

tests, surgical therapy, postoperative course, autopsy findings, and follow-up. Information obtained from the registry was supplemented with direct chart review. Statistical analysis was by χ^2 and Mann-Whitney U test where appropriate. A p value of < 0.05 was considered significant.

Results

Sixty-five cases of congenital diaphragmatic hernia were discovered in 241,473 births, for an incidence of 1 in 3715. Ninety-two percent of these cases were Bochdalek hernias (78% left, 14% right), 1.5% were Morgagni hernias, and 1.5% involved complete absence of the diaphragm. The remaining 5% were classified only as "congenital."

Eighteen infants (18/65, 28%) had associated severe abnormalities (Table I). All could potentially have been identified prenatally by ultrasonography or karyotype analysis. Four of these 18 pregnancies (22.2%) were complicated by polyhydramnios. The survival rate of infants with congenital diaphragmatic hernia associated with other malformations was 5.5%. Fourteen infants were stillborn and 3 died shortly after birth. One infant with severe microcephaly survived and at 28 months had a seizure disorder, orthopedic problems, esotropia, and severe developmental delay.

In an effort to focus on potentially salvageable infants, the 17 infants whose deaths were related to associated severe malformations and the one surviving infant with severe microcephaly (who might not have been a candidate for antenatal surgery) were then excluded from the study. The remaining 47 infants were grouped according to survival (n = 26) versus nonsurvival (n = 21), and their characteristics were compared. No significant differences were found between the groups with respect to maternal age, gravidity, parity, gestational age at delivery (one preterm delivery in each group), birth weight, infant sex, hernia type, record of prenatal ultrasonography, family history of genetic problems, or pregnancy complications such as preterm labor, premature rupture of membranes, preeclampsia,

diabetes, hypertension, seizure disorder, twins, or placenta previa. The incidence of polyhydramnios was not significantly different between the groups. Five pregnancies were complicated by polyhydramnios (none of these infants were delivered preterm); one infant lived.

The cesarean section rates of infants with isolated congenital diaphragmatic hernia were similar for both groups (31% survival vs 42% nonsurvival, p = 0.72). The groups were equally likely to be delivered in a level I or level II hospital (88.5% survival vs 76% nonsurvival, p = 0.47) and to be transferred to a level III hospital (56% survival vs 72% nonsurvival, p = 0.56). One characteristic associated with a poor prognosis was the need for aggressive resuscitation of the infant at delivery (27% survival vs 100% nonsurvival, p = 0.007).

All infants with isolated congenital diaphragmatic hernia except three (in the nonsurvival group) underwent postnatal surgery. No infant received extracorporeal membrane oxygenation. Only two surviving infants, compared with 19 nonsurvivors, had severe pulmonary and cardiac problems (pulmonary hypoplasia, pulmonary hypertension, persistent fetal circulation, and dextrocardia) that were related to the hernia (p < 0.0001). Death was attributed to hernia-associated problems in 19 cases (40% of infants with isolated congenital diaphragmatic hernia) and to postoperative complications in the remaining two cases.

We attempted to determine whether the level of the delivery hospital influenced outcome. All cases were grouped according to delivery in level I or II hospitals (group 1, n = 49) or level III hospitals (group 2, n = 16; Table II). Patients in group 1 were significantly more likely to have had uncomplicated pregnancies. They were significantly less likely to have had the hernia identified antenatally by ultrasonography or to have had infants with other malformations. The survival rate between the two groups was similar.

The survival rate for infants with isolated diaphragmatic hernia was 55% (26/47). Specific long-term follow-up information was available on 13 infants (range,

	Group 1 $(n = 49)$		$Group \ 2 \ (n = 16)$		
	No.	%	No.	%	p Value
No ultrasonography					
Normal pregnancy	32	65.3	1	6.2	0.0003
Prenatal ultrasonography			,		
Hernia identified	3	6.1	9	. 56.2	0.0000
Hernia not identified	7	14.3	l	6.2	0.681
Complicated pregnancy	7	14.3	5	31.2	0.251
Other malformations	9	18.4	9	56.2	0.007
Survivors	23	46.9	4	25.0	0.210

Table II. Prenatal history and outcome according to level of delivery hospital

Group 1, Delivery at level I or II hospitals. Group 2, Delivery at level III hospitals.

3 to 48 months). The condition of 12 infants was described as excellent. One infant had seizures and developmental delay at 19 months.

Comment

The diaphragm develops from four separate structures (the septum transversum, the pleuroperitoneal membranes, the dorsal mesentery of the esophagus, and the body wall) that grow toward each other and fuse by the end of the eighth week of life. Failure of one of these structures (usually the pleuroperitoneal membranes) to grow or fuse results in a defect in the diaphragm, which allows free communication between the thoracic and abdominal cavities. When abdominal organs enter the chest through such a defect, lung development may be impeded, the heart may be displaced, and vascular structures may be distorted. It is thought that these alterations result in pulmonary hypoplasia, pulmonary hypertension, and cardiac abnormalities, although this has not been clearly demonstrated. When these abnormalities are present, they cause significant neonatal morbidity and mortality and may preclude recovery in spite of the best surgical correction at birth. Attempts have been made to define prognostic indicators of outcome, but such indicators generally have been limited to neonatal parameters.8-10 There is as yet no clear evidence that prognosis can be defined antenatally, although polyhydramnios has been associated with a poor outcome.3.4

Mortality rates associated with congenital diaphragmatic hernia have been reported to range from 24% to 95%.3,4,7,11,12 This spectrum of risk probably results from the various methods used to obtain data and from the inconsistent inclusion or exclusion of fetuses who have other malformations. The association of congenital diaphragmatic hernia with additional severe malformations in a large proportion of patients (29% to 53%) has been described, 4, 5, 11, 13 underlining the heterogeneous pathogenesis of this defect. The association of congenital diaphragmatic hernia with schisis defects, trisomies, and certain well-defined conditions such as Cornelia de Lange syndrome emphasizes the pleiotropy

of causal factors contributing to congenital diaphragmatic hernia. Because congenital diaphragmatic hernia can arise from and be associated with a variety of pathologic processes, it is not surprising that prediction of outcome has been difficult.

When other major malformations are present in association with congenital diaphragmatic hernia, they are usually directly responsible for perinatal death. Studies investigating the postnatal management of infants with congenital diaphragmatic hernia indicate that only those infants with isolated congenital diaphragmatic hernia are candidates for postnatal surgery or other therapy. When antenatal corrective surgery for such fetuses is considered, only morbidity and mortality rates from similarly affected fetuses should be used for comparison. Accurate morbidity and mortality rates from fetuses with isolated congenital diaphragmatic hernia have been previously unavailable.

Existing prevalence and outcome studies are largely flawed by biased or inadequate data collection. One study that purported to reveal the "hidden mortality" associated with congenital diaphragmatic hernia⁷ used data obtained from one major referral center and from an infant death registry. Live-born infants with congenital diaphragmatic hernia who were not referred to that center were undetected. In addition, information regarding associated anomalies, which were expected in 29% to 53% of cases, was not available for more than half the study patients. It is possible that the infants referred to the major center for surgery had isolated congenital diaphragmatic hernia, while those who either had died before transfer or were not transferred had associated malformations and would not have been surgical candidates. It is also possible that, as in certain cases in our study, some children with isolated congenital diaphragmatic hernia were successfully cared for locally. The conclusion reached by those authors, which is that the "true" mortality of congenital diaphragmatic hernia is 66%, cannot be substantiated without additional data.

Another study obtained information about outcome in congenital diaphragmatic hernia from a physician survey.3 In spite of the bias inherent in this method of data collection, the mortality rate of 80% suggested by that article has been used to justify prenatal surgery.2 This rate, however, included 15 fetuses with associated severe malformations who would not have been surgical candidates. Similar inclusion of fetuses with multiple malformations in another series yielded a mortality rate of 90.5%.4 Although that series was biased by data collection techniques (all cases were cared for at one referral center), 9/19 fetuses (47%) in this series had associated severe malformations that would have precluded surgical therapy. Only a complete population survey in which all infants born with congenital diaphragmatic hernia are identified and evaluated for . associated anomalies will yield accurate prognostic data about the outcome for fetuses with this defect.

Because the Iowa Birth Defects Registry identifies all infants who are born with a congenital malformation at >20 weeks' gestation or who weigh >500 gm, stillbirths, neonatal deaths, and surviving infants are identified regardless of physician, hospital of birth, or subsequent treatment. Our estimated prevalence of 1 in 3715 is similar to that reported by a large British study (1/3030)14 and is actually higher than the 1 in 5455 reported by Harrison et al.7

Twenty-eight percent of infants in our series had associated severe malformations, the spectrum of which was similar to that reported in other series. All were theoretically prenatally identifiable by means of ultrasonography or karyotype analysis, and none of those infants would have been candidates for prenatal surgery. Cases such as these must be excluded from statistical analysis when the background mortality rate for congenital diaphragmatic hernia is used as a rationale for antenatal therapy.

The survival rate among the 47 cases of isolated congenital diaphragmatic hernia in our survey was 55%. Eighty-five percent (40/47) were delivered in level I or II hospitals, and 43% underwent surgery for repair at a level I or II hospital. None was treated with extracorporeal membrane oxygenation. The choice of delivery hospital was probably related to many factors but was undoubtedly strongly influenced by the high incidence of apparently uncomplicated pregnancies and by the lower rate of prenatal detection of the hernia in the group delivered at level I or II hospitals. Because routine obstetric ultrasonography was not common at the time of this study (and others), it is possible that the infants with prenatal diagnosis and referral to a tertiary care center for delivery were those with the most antenatal complications and therefore the worst prognoses. The infants whose gestations were relatively uncomplicated appeared to have a similar survival rate in spite of delivery at non-tertiary-level hospitals, although this lack of a statistical difference may represent a type II error. Recent data suggest that survival among infants with isolated congenital diaphragmatic hernia can be increased from 50% to 76% with the use of extracorporeal membrane oxygenation and other aggressive postdelivery management. 12, 15 It is likely that some of the nonsurvivors who were delivered at level I or II hospitals may have benefited from this treatment. As antenatal ultrasonography becomes more common, similar infants with congenital diaphragmatic hernia and otherwise uncomplicated pregnancies will be identified much more often before delivery. Antenatal diagnosis as the serendipitous result of routine ultrasonography probably does not have the same prognostic significance as does diagnosis made during the evaluation of polyhydramnios or other pregnancy complications. The temptation to consider antenatal surgery in these salvageable infants will be strong, but these infants are the ones most likely to respond to aggressive neonatal management and they are the most likely to survive without in utero surgery.

Our study also emphasizes the skewed data that are likely to be generated from patients referred to a tertiary care center. The distribution of cases in our series suggests that such centers see the most compromised fetuses with the worst prognoses. Fetuses with relatively uncomplicated congenital diaphragmatic hernia are more likely to be cared for locally and without the aid of specialty consultants.

Finally, it is possible that there is a subset of infants with isolated congenital diaphragmatic hernia who would benefit from antenatal surgery. In our series 40% of infants with the isolated defect died because of hernia-associated problems. If these infants could somehow be distinguished antenatally, they would be the most logical candidates for surgery. Presently, this determination is not possible.

In summary, our population-based study determined that the survival rate for infants with isolated congenital diaphragmatic hernia was 55%. If these infants had been identified prenatally, referred to a tertiary care center, and aggressively managed at birth, their survival rates might have been even better. In utero surgery probably has a future, but it should be reserved for fetuses whose anomalies predict lethal outcomes if not repaired antenatally. Those fetuses with congenital diaphragmatic hernia who would benefit from such surgery need to be clearly identified, and techniques must be developed to distinguish them from their lower-risk cohorts before antenatal repair is offered.

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Intrapartum ultrasonographic estimates of fetal weight by the house staff

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In spite of the widespread use of ultrasonographic estimates of fetal weight, a paucity of data exists with regard to its use in patients who are in labor. The purpose of this study was to evaluate the accuracy of ultrasonographic estimates of fetal weight in a busy labor and delivery suite by the house staff.

Measurements of biparietal diameter, abdominal circumference, and femur length were prospectively obtained in 109 patients in labor in whom this information was expected to be contributory in making delivery plans. All patients were delivered within 48 hours of ultrasonographic evaluation. Measurements of abdominal circumference were obtained in all cases, Biparietal diameter and femur length were obtained in 85% and 92% of cases, respectively. Overall, the mean absolute errors were 9.3% and 9.2% for estimated fetal weight by biparietal diameter/abdominal circumference/femur length ratios, respectively. Estimated fetal weight by biparietal diameter/abdominal circumference ratio was not significantly different from that by femur length/abdominal circumference ratio. In conclusion, the accuracy of intrapartum estimates of fetal weight performed by the house staff in a busy labor and delivery unit is comparable to that reported for estimates obtained during the antepartum period by professional users of ultrasonography in a controlled setting. (AM J OBSTET GYNECOL 1991;165:842-5.)

Key words: Estimated fetal weight, ultrasonography, house staff

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Ultrasonography, since its introduction in the 1960s, has provided a "window" into the uterus. Early technology enabled the evaluation of fetal number and the site of placentation. With the advent of gray-scale ultrasonography fetal structure was more easily delineated, and it became possible to diagnose morphologic abnormalities antenatally. In addition, measurements

of head, trunk, abdomen, and extremities have established a set of norms for gestational age that is indispensable for accurate dating and assessment of adequate growth in utero. Real-time ultrasonography has provided the ability to assess the fetus in its own minuteto-minute environment. Observations of fetal activity and response to various stimuli have created new insight into the universe of the developing human.

In the 1970s investigators proposed using ultrasonography to obtain estimates of fetal weight. A variety of formulas were evaluated, including cephalometry, serial parallel scans of the entire length of the fetus, thoracic and abdominal circumferences, diameters or cross-sectional areas, and various combinations of these measurements.14

The formulas most often used are logarithmic derivatives of multivariate regression analysis that are based on biparietal diameter and abdominal circumference⁵ and on abdominal circumference and femur length.6 These formulas became popular because of the ease in obtaining these measurements and the accuracy of the estimate generated. Both models report a systematic error of ≤10% relative to the actual birth weight.

A precise estimate of fetal weight may be an important factor in the evaluation of patients in labor. Accurate clinical estimate of fetal size by palpation of the abdomen is a function of the experience of the examiner and can be influenced by the size and shape of the patient. It has been shown that clinical estimates of fetal weight are most inaccurate in the extremes of fetal weight—very large or very small fetuses.7.8

Most studies of the accuracy of ultrasonographic fetal weight estimates have had the measurements performed by professional users of ultrasonography in a laboratory setting. In many institutions, however, a trained ultrasonographer is not always available at the time management decisions need to be made. In addition, a bedside sonogram obtained from a patient in active labor can be technically challenging.

In a busy inner-city hospital with a resident-run labor floor, decisions are often made on the basis of an ultrasonographic scan obtained by the admitting resident. It is important to know how accurate these estimates are and how much they can be relied on in the care of the patient. Therefore the purpose of this study was to evaluate the accuracy of ultrasonographic estimates of fetal weight performed by residents in an acute-care setting.

Material and methods

This study was conducted prospectively from January through July 1990 on the labor floor of the Bronx Municipal Hospital Center. Ultrasonographic evaluation was performed by the admitting resident whenever

Table I. Indications for testing

	No.	%
Premature rupture of membranes	19	17.4
Preterm labor	15	13.7
Suspected macrosomia	14	12.8
Small for dates	10	9.2
Uncertain dates	11	10.2
Vaginal bleeding	14	12.8
Breech	12	11.0
Twins	6	5.5
Other	8	7.4
TOTAL	109	100

it was thought that an ultrasonographic estimate of fetal weight would aid in the management of labor. Only patients with a live fetus, with no known anomalies, and in active labor were included in this study.

Ultrasonographic scans were performed by thirdand fourth-year residents using the Ultramark IV (ATL, Inc., Tempe, Ariz.) dynamic image scanner with a 3.5 MHz linear transducer. Electronic caliper measurements of the biparietal diameter, abdominal circumference, and femur length were made with the use of defined landmarks. Estimates of fetal weight were calculated by computer-generated regression equations as per Shepard et al.5 and Hadlock et al.6 The mean and absolute error percentages defined as

$$\frac{\text{(Birth weight - Estimated weight)}}{\text{Birth weight}} \times 100$$

were used for comparison of estimates.

Results

A total of 109 patients were entered in the study and 115 sets of measurements were obtained (i.e., 103 singleton gestations and six sets of twins). All patients were delivered within 48 hours, 76 (60.3%) within 12 hours and 93 (85%) within 24 hours. Birth weight (mean \pm SD) was 2701 \pm 915 gm.

The leading indications for ultrasonographic evaluation were fetal growth abnormalities, premature rupture of the membranes, and preterm labor representing 22%, 17.4%, and 13.7% of measurements, respectively (Table I).

Measurement of the abdominal circumference was obtained for all fetuses. In 16 patients (15%) a biparietal diameter could not be obtained because of technical difficulties. Similarly, femur length was not obtained in nine (8%) fetuses scanned. The inability to obtain a biparietal diameter or a femur length was distributed throughout the different indications for testing, with somewhat fewer measurements of both biparietal diameter and femur length in patients with suspected macrosomia (five of 25 absent measurements) and uncertain dates (6/25).

A total of 205 estimates were generated (99 estimates

Table I	. Distrib	ution of	absolute	error e	of 115	fetal	weight	estimates
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Estimated fetal weight	Within 5% of birth weight (%)	Within 10% of birth weight (%)	Within 15% of birth weight (%)	>20% of birth weight (%)
By biparietal diameter/abdominal circumference	35.7	60	78.5	10.2
By abdominal circumference/femur length	39	60.9	81	10.5

Table III. Mean absolute error (%)

	Birth weight				
Estimated fetal weight	<2500 gm (%)	2500-4000 gm (%)	>4000 gm (%)		
By biparietal diameter/abdominal circumference	$ \begin{array}{c} 10.8 \\ (n = 40) \end{array} $	8.7 $(n = 50)$	7.1 $(n = 8)$		
By abdominal circumference/femur length	$9.6 \\ (n = 43)$	9.3 $(n = 53)$	$ \begin{array}{c} (n = 9) \\ \end{array} $		

by biparietal diameter/abdominal circumference, and 106 estimates by femur length/abdominal circumference). Sixty percent of these estimates were within 10% of the actual birth weights for each of these methods. The estimates were within 5% of birth weight in 35.7% of those calculated from biparietal diameter/abdominal circumference and in 39% of those calculated with abdominal circumference/femur length. These differences were not significant (Table II).

The mean errors for estimated fetal weight by biparietal diameter/abdominal circumference and femur length/abdominal circumference were 9.3% and 9.2%. There was no statistical difference between the two formulas. The mean overall error was 10.1% in the fetuses whose birth weights were <2500 gm, 9.0% in the fetuses whose birth weights were between 2501 and 4000 gm, and 7.0% in the fetuses whose birth weights were >4000 gm (Table III).

The birth weight was <1500 gm in 13 patients who were enrolled in this study. The mean birth weight was 1046 gm (range, 590 to 1390 gm). The mean estimated fetal weight calculated by biparietal diameter/abdominal circumference was 1033 gm (range, 535 to 1452 gm), and the mean error was 10.9%.

Estimates of fetal weight were obtained in 19 fetuses with a diagnosis of premature rupture of membranes. The mean errors were 10.5% for estimated fetal weight by biparietal diameter/abdominal circumference and 9.4% by abdominal circumference/femur length.

Comment

There have been numerous studies investigating the accuracy of the ultrasonographic methods of estimating fetal weight. Most investigators report a mean absolute error ranging between 8.0% and 13.8% of birth weight. The results of this study indicate that a similar mean absolute error (9.3%) can be obtained by the house staff

in an acute-care setting. The mean absolute error for the very-low-birth-weight babies was comparable to that seen for the general population.

The estimated fetal weight by biparietal diameter/abdominal circumference was reported separately by Warsof et al., Timor-Tritsch et al., and Shepard et al. Their studies report a range of 54% to 72.5% of estimated fetal weights that were within 10% of the actual birth weights. This is comparable to the 60% of patients in our study who had estimated fetal weights within 10% of birth weights.

The studies of Hadlock et al.⁶ and Yarkoni et al.¹¹ show a mean error for the use of estimated fetal weight by femur length/abdominal circumference measurements that is ≤4%. The results of our study show a similar accuracy with a mean error of 1.2%.

Most studies of estimated fetal weight have been conducted in the antepartum period. Ultrasonographic study of patients in labor presents some unique problems, including both patient discomfort caused by uterine contractions and the operator's discomfort caused by the contorted position necessary for him or her to perform a bedside ultrasonographic examination. It has been suggested that measurement of the biparietal diameter in labor may be unreliable or unobtainable because of fetal skull engagement and molding. ¹² Our findings suggest that only a small percentage (15%) of measurements of biparietal diameter could not be obtained in this study population.

Of interest are the patients scanned with premature rupture of membranes. Other studies report an underestimation of fetal weight with oligohydramnios associated with rupture of membranes. This was not reflected in this study, where the error was 10.5% for estimated fetal weight by biparietal diameter/abdominal circumference and 9.4% for estimated fetal weight by abdominal circumference/femur length; however,

this subgroup of our patient population was relatively

In conclusion, the results of this study indicate that the accuracy of intrapartum estimates of fetal weight by the house staff in a busy labor and delivery unit is comparable to that reported by skilled operators in more controlled settings.

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The cheek-to-cheek diameter in the ultrasonographic assessment of fetal growth

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Deviations from normal growth modify the amounts of adipose tissue and muscle mass in the adult and the fetus. As an index of the amount of adipose tissue in the fetus, the cheek-to-cheek diameter obtained on an ultrasonographic coronal view of the face at the level of the nostrils and lips was evaluated. Two hundred thirty-four singleton uncomplicated, well-dated pregnancies ranging from 20 to 41 weeks were studied. Two hundred fetuses with biometric measurements or estimated weights between the 10th and 90th percentile for gestational age were included in obtaining a nomogram. Cheek-to-cheek diameter as a function of gestational age was expressed by the regression equation: Cheek-to-cheek diameter (centimeters) = -0.908 + 0.195 Gestational age (gestational age ≥ 20 weeks), with a Pearson correlation coefficient of $R^2 = 0.806$. The cheek-to-cheek diameter/biparietal diameter ratio was almost constant, independent of gestational age, and ranged from 0.6 at 20 weeks to 0.7 at 41 weeks. Fetuses with intrauterine growth retardation tended to have lower ratios. Macrosomic fetuses of diabetic mothers had larger cheek-to-cheek diameters and elevated ratios, whereas large-for-gestational-age fetuses of nondiabetic mothers had normal ratios. (AM J Obstet Gynecol 1991;165:846-52.)

Key words: Ultrasonography, fetal growth, nomogram, cheek-to-cheek diameter

Multiple ultrasonographic parameters have been used to evaluate fetal growth and nutritional status with varying degrees of success. In infants, measurements of subcutaneous fat in the flank, forearms, or thighs are currently used as clinical indexes for signs of deviation from normal growth. 2.3

It is common knowledge that poor nutritional status is reflected by sunken cheeks, whereas individuals with increased weight will have an overall larger deposition of adipose tissue in their bodies, and in their cheeks in particular. Studies in which fetal subcutaneous tissues have been evaluated by radiography and correlated to weight have revealed retarded fetal growth to be associated with decreased fat thickness.⁴

Our study was designed to indirectly evaluate by ultrasonography amounts of subcutaneous adipose tissue in fetuses by measuring the distance between the cheeks (cheek-to-cheek diameter) in uncomplicated gestations and to establish a nomogram of the cheek-to-cheek diameter in relation to gestational age. Furthermore, we examined the validity of the measurements in a small

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sample of fetuses with abnormal growth (intrauterine growth retardation [IUGR] or macrosomia).

Material and methods

Two hundred thirty-four patients referred to Strong Memorial Hospital, the regional perinatal center, for routine ultrasonography were examined with commercially available real-time equipment with 3.5 or 5 MHz transducers (AI 5200, Acoustic Imaging, Phoenix, Ariz.; ATL Ultramark 9, ATL, Bothell, Wash.; Aloka 650, Aloka 280, Corometrics Medical Systems, Inc., Wallingford, Conn.). Only uncomplicated singleton pregnancies were included. Gestational age was obtained from a well-defined last menstrual period with confirmation by early ultrasonography, before 20 weeks. Gestational age ranged from 20 to 41 weeks. Fetal measurements included cheek-to-cheek diameter, biparietal diameter, head circumference, abdominal circumference, and femur length. The cheek-to-cheek diameter was obtained on a coronal view of the fetal face at the level of the nostrils and lips (Figs. 1 and 2). Very rarely, because of shadowing, the distal cheek could not be clearly visualized. In these cases the distance from the proximal cheek to the middle of the mouth was measured and multiplied by two (Fig. 3). This was used in only five cases in the derivation of the regression equation. Estimated fetal weight was obtained from biparietal diameter and abdominal circumference measurements.5 Only fetuses between the 10th and 90th percentile by biometry or estimated fetal weight were included in establishing a nomogram

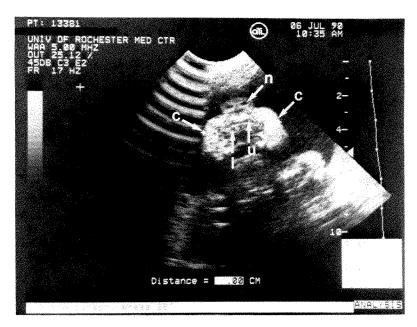


Fig. 1. Coronal view of fetal face at $27\frac{1}{2}$ weeks' gestation. Nostrils (n), lips (upper, u; lower, l), and cheeks (c) are evident. Markers represent cheek-to-cheek diameter.

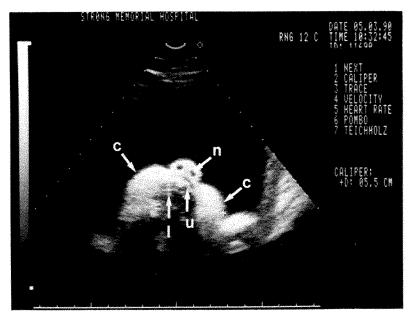


Fig. 2. Cheek-to-cheek diameter at 31% weeks' gestation. Nostrils (n), lips (upper, u: lower, l), and cheeks (c) are evident. Markers represent cheek-to-cheek diameter.

(N = 200). All deliveries occurred after 37 completed weeks, and no infants had evidence of growth disturbance (IUGR or macrosomia) or congenital anomalies. One measurement per fetus was recorded, thus providing cross-sectional data. Twenty fetuses had multiple measurements for interobserver and intraobserver error analysis: After a cheek-to-cheek diameter was obtained by one observer, the picture was frozen and measurements were taken. A second observer then performed measurements on the frozen image without knowledge of the previously obtained value. The picture was then unfrozen and a new cheek-to-cheek diameter was selected. A hard copy was then taken and measurements were performed by two independent observers. An evaluation was also made of the feasibility of the technique by trying to obtain a cheek-to-cheek diameter for every single obstetric ultrasonography performed between 20 and 41 weeks' gestational age during a 1-week period. Measurements were difficult to obtain before 20 weeks, but this probably is not of



Fig. 3. Distal cheek is not clearly visualized. Measurement of one-half cheek-to-cheek diameter at 34% weeks' gestation. *Central marker* lies beneath fetal nasal septum.

Table I. Cheek-to-cheek diameter predicted by gestational age (mean ± 2 SD)

	Cheek-to-cheek diameter (cm, rounded to nearest mm)			
Gestational age (wk)	-2 SD	Mean	+ 2 SD	
20	2.0	3.0	4.0	
21	2.2	3.2	4.2	
22	2.3	3.4	4.4	
23	2.5	3.6	4.6	
24	2.7	3.8	4.8	
25	2.9	4.0	5.0	
26	3.1	4.2	5.2	
27	3.3	4.4	5.4	
28	3.5	4.6	5.6	
29	3.7	4.7	5.8	
30	3.9	4.9	6.0	
31	4.1	-5.1	6.2	
32	4.3	5.3	6.4	
33	4.5	5.5	6.6	
34	4.7	5.7	6.8	
35	4.9	5.9	6.9	
36	5.1	6.1	7.1	
37	5.3	6.3	7.3	
38	5.5	6.5	7.5	
39	5.7	6.7	7.7	
40	5.9	6.9	7.9	
41	6.1	7.1	8.1	

primary clinical importance because the fetus gains 95% of its weight in the last 20 weeks of gestation.

The data were analyzed with the SAS and Minitab programs. Correlation between cheek-to-cheek diameter and biparietal diameter, estimated fetal weight, and gestational age were studied with Pearson correlation coefficients. Regression analysis was performed,

and the best-fit equations for cheek-to-cheek diameter and the cheek-to-cheek diameter/biparietal diameter ratio as a function of gestational age and gestational age as a function of cheek-to-cheek diameter were obtained. Confidence intervals corresponding to ± 2 SD (i.e., 95% of the population; 2.5% being above and 2.5% below normal limits) were established.

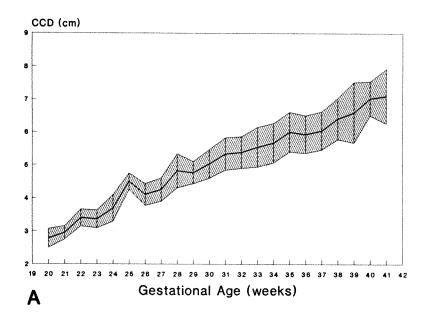
After nomograms were derived, measurements of cheek-to-cheek diameter and cheek-to-cheek diameter/biparietal diameter ratios in large-for-gestationalage (LGA) fetuses (biometry or estimated fetal weight, >90th percentile) and small-for-gestational-age (SGA) fetuses (biometry or estimated fetal weight, <10th percentile) were obtained for comparison purposes. LGA fetuses were separated into those of diabetic or non-diabetic mothers. Another category examined was appropriate-for-gestational-age (AGA) fetuses of diabetic mothers.

Results

The growth of the cheek-to-cheek diameter during pregnancy was found to be linear from 20 to 41 weeks. The linear regression equation as function of gestational age was:

$$CCD = -0.908 + 0.195 \text{ GA } (GA \ge 20 \text{ weeks})$$

where CCD is cheek-to-cheek diameter in centimeters and GA is gestational age in weeks. The coefficient of correlation (Pearson) was $R^2 = 0.806$. Table I represents predicted cheek-to-cheek diameters at different gestational ages, including mean \pm 2 SD. Fig. 4 is the graphic representation of these data. The upper part



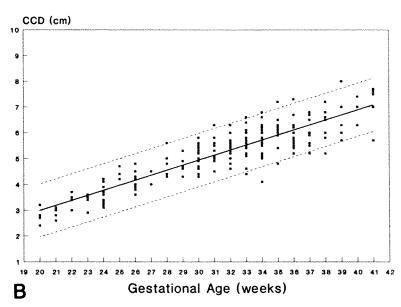


Fig. 4. Cheek-to-cheek diameter as a function of gestational age. **A,** Observed mean \pm 2 SD. **B,** Predicted mean by regression equation \pm 2 SD. *Squares,* Individual data pairs.

represents the observed mean (± 2 SD) and the lower part, the equation-predicted mean ± 2 SD with the scattergram of data pairs superimposed. The equation of gestational age as a function of cheek-to-cheek diameter was:

$$GA = 9.77 + 4.16 CCD$$

where CCD is cheek-to-cheek diameter and GA is gestational age (with the same coefficient of correlation). Table II represents the predicted gestational age with the mean \pm 2 SD, as derived from the cheek-to-cheek diameter for selected values. Pearson correlation coefficients (R^2) for cheek-to-cheek diameter as a function of gestational age, biparietal diameter, and estimated

fetal weight were 0.807, 0.756, and 0.767, respectively. The regression equation for the cheek-to-cheek diameter/biparietal diameter ratio as a function of gestational age was:

CCD/BPD =
$$0.47 \pm 0.0066$$
 GA (GA ≥ 20 weeks)

where CCD is cheek-to-cheek diameter and GA is gestational age.

Therefore the ratio ranged from 0.6 at 20 weeks to 0.74 at 41 weeks (mean ± 2 SD = 0.68 ± 0.1), i.e., gestational age has very little influence on the ratio in normal pregnancies within the above-mentioned gestational age. Table III and Fig. 5 represent the normal values and the diagrammatic representation of the data

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Table II. Gestational age predicted by cheek-to-cheek diameter (mean ± 2 SD)

	Gestational age (wk and day)			
Cheek-to-cheek diameter	-2 SD	Mean	+2 SL	
2.5	15 + 3	20 + 1	25 + (
2.7	16 + 2	21 + 0	25 + 5	
3.0	17 + 3	22 + 2	27 + 6	
3.2	18 ± 2	23 + 1	27 + €	
3.5	19 + 4	24 + 2	29 + 1	
3.7	20 + 3	25 + 1	30 ± 6	
4.0	21 ± 5	26 + 3	31 + 1	
4.2	22 + 3	27 + 2	32 ± 0	
4.5	23 + 5	28 ± 3	33 + 2	
4.7	24 + 4	29 + 2	34 + 1	
5.0	25 + 6	30 ± 4	35 + 2	
5.2	26 ± 5	31 + 3	36 ± 1	
5.5	27 + 6	32 + 5	37 + 3	
5.7	28 ± 5	33 + 3	38 + 2	
6.0	30 ± 0	34 + 5	39 ± 4	
6.2	30 + 5	35 ± 4	40 + 2	
6.5	32 ± 0	36 + 6	41 + 4	
6.7	32 + 6	37 ± 5	42 + 3	
7.0	34 + 1	38 ± 6	43 + 5	
7.2	35 + 0	39 ± 5	44 + 3	
7.5	36 + 1	41 + 0	45 + 5	

Table III. Cheek-to-cheek diameter/biparietal diameter ratio predicted by gestational age (mean \pm 2 SD)

	Cheek-to-cheek diameter/biparietal diameter			
Gestational age (wk)	-2 SD	Mean	+ 2 SD	
20	0.46	0.60	0.74	
21	0.47	0.61	0.75	
22	0.48	0.62	0.76	
23	0.48	0.62	0.76	
24	0.49	0.63	0.77	
25	0.50	0.64	0.78	
26	0.50	0.64	0.78	
27	0.51	0.65	0.79	
28	0.52	0.66	0.80	
29	0.52	0.66	0.80	
30	0.53	0.67	0.81	
31	0.54	0.68	0.82	
32	0.54	0.68	0.82	
33	0.55	0.69	0.83	
34	0.56	0.70	0.84	
35	0.56	0.70	0.84	
36	0.57	0.71	0.85	
37	0.57	0.72	0.86	
38	0.58	0.72	0.86	
39	0.59	0.73	0.87	
40	0.60	0.74	0.87	
41	0.60	0.74	0.88	

for the cheek-to-cheek diameter/biparietal diameter ratio (upper and lower part, as in Fig. 4).

The cheek-to-cheek diameters of 10 fetuses with IUGR were compared with those of 200 normal fetuses

by t test for unpaired data. A trend was demonstrated for smaller cheek-to-cheek diameters in IUGR, but numbers were too small to attain statistical significance (p=0.74). The same held true for the cheek-to-cheek diameter/biparietal diameter ratio (p=0.8). Mean values of the cheek-to-cheek diameter and the cheek-to-cheek diameter/biparietal diameter ratios for LGA fetuses are shown in Table IV. Cheek-to-cheek diameters of LGA fetuses were consistently larger than those of AGA fetuses, with LGA fetuses of diabetic mothers having very significantly larger cheek-to-cheek diameters and cheek-to-cheek diameter/biparietal diameter ratios than all other fetuses. Measurements in AGA fetuses of diabetic mothers were not different from fetuses of nondiabetic mothers (controls).

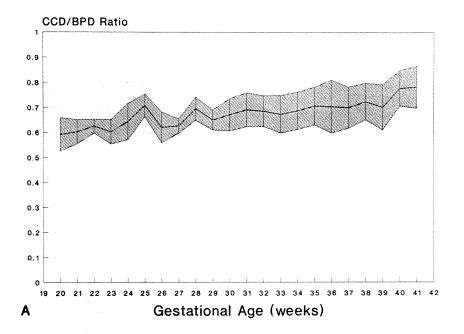
Cheek-to-cheek diameter was obtained in 70% of cases. Only when the fetus was directly occipitoanterior (face down) was it impossible to obtain the appropriate image. Often waiting a few minutes or having the patient return after a short interval permitted the appropriate visualization.

Interobserver and intraobserver error calculation demonstrated the reproducibility of the method, with both observers being reliable and their results not statistically different from each other on the same image or comparing images obtained separately at different time intervals. (Paired t test p values for interobserver and intraobserver variability were 0.32 and 0.58, respectively).

Comment

Our results demonstrate that in normal pregnancies the cheek-to-cheek diameter is directly correlated with gestational age. The cheek-to-cheek diameter/biparietal diameter ratio is almost age independent in fetuses with normal growth, varying from 0.6 to 0.74 between 20 and 41 weeks' gestation.

Multiple fetal organs have been measured by ultrasonography to follow growth or weight or to evaluate gestational age, from the early biparietal diameter more than 20 years ago to fetal long bones and, recently, clavicles. Most measurements involve bony structures or body parts as a reflection of the growth of the internal organs (abdominal or chest circumference, for instance). In infants the adequacy of intrauterine nutrition has been evaluated by measurements of the arm circumference³ or skin-fold thickness.⁸ Thigh circumference has been used as an index of amounts of soft tissues to estimate fetal growth.9 Subcutaneous fat has been observed radiographically in the fetus and has been correlated with weight. Jeanty et al.2 demonstrated that limb volume is a function of the amount of adipose tissue and is strongly correlated with gestational age. However, these authors point out that because of technical limitations and overlapping values of



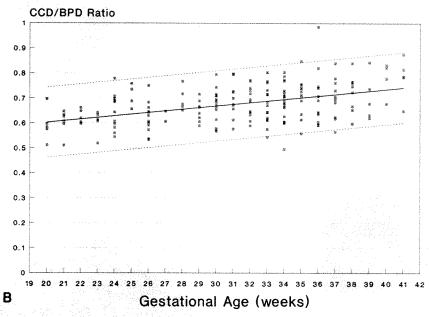


Fig. 5. Cheek-to-cheek diameter/biparietal diameter as function of gestational age. A, Observed mean \pm 2 SD. B, Predicted mean by regression equation \pm 2 SD. Squares, Individual data pairs.

Table IV. Measurements in fetuses of diabetic mothers and LGA fetuses (mean and SD)

	Cheek-te	o-cheek diameter (c	m)	Cheek-to-cheel	t diametèr/biparieta ratio	l diameter
Condition	Actual mean and SD	Expected mean and SD	p Value	Actual mean and SD	Expected mean and SD	p Value
LGA diabetic mother $(n = 10)$ (33.3 wk*)	6.37 (0.75)	5.58 (0.60)	0.0001	0.77 (0.07)	0.69 (0.07)	0.01
AGA diabetic mother $(n = 9)$ (35.4 wk*)	6.19 (0.89)	5.99 (0.60)	0.6	0.73 (0.04)	0.70 (0.07)	0.61
LGA nondiabetic $(n = 29) (30.9 \text{ wk*})$	5.44 (0.63)	5.11 (0.54)	0.0058	0.68 (0.08)	0.67 (0.06)	0.77

^{*}Mean gestational age.

subcutaneous tissue thickness, this measurement is a poor predictor of growth and is useful only in extreme cases.

Fetal cheeks have been previously identified by ultrasonography with clear visualization of the anatomically defined corpus adiposum buccae, Bichat's fatpad.¹⁰ This is the area that we chose to measure to obtain the cheek-to-cheek diameter, because it is mainly formed of white fat that is sensitive to changes in nutritional status.6 Excessive body size is caused by skeletal frame enlargement and, to a larger degree, by more abundant body fat mass. This is particularly demonstrated in infants of diabetic mothers.8 Maternal diabetes induces accelerated fetal growth in insulin-sensitive tissues. 6, 11 Because the brain is not insulin-sensitive, cephalic growth is not affected. Somatic growth of the fetal abdomen and other body areas with subcutaneous adipose tissue is affected. Therefore the macrosomic infant of a diabetic mother has an asymmetrically large body in relation to head size. These infants are "bulkier" than infants of the same weight born to nondiabetic mothers. 12, 13 The detection of fetal macrosomia is possible by ultrasonography. 12 Recently, macrosomia in the infant has been described as different, depending on whether the mother is diabetic,13 with certain indexes, such as femur length/abdominal circumference ratio, being very helpful in the differentiation.14 This concept has been challenged by authors,15 as has the clinical usefulness of the femur length/abdominal circumference ratio in predicting macrosomia in fetuses of diabetic mothers.16 At the other side of the scale, gestational age-independent indices are also needed to identify SGA fetuses.

In our study, mean values of the cheek-to-cheek diameter were larger in LGA than in AGA fetuses, particularly in LGA fetuses of diabetic mothers. LGA fetuses of diabetic mothers could be differentiated by their having a cheek-to-cheek diameter/biparietal diameter ratio significantly larger than controls, while the ratio in LGA fetuses of nondiabetic mothers fell in the normal range. In our small sample of IUGR fetuses, the cheek-to-cheek diameter and the cheek-to-cheek diameter/biparietal diameter ratio demonstrated a trend toward being smaller, suggesting a reduction in the amount of subcutaneous fat in the fetal cheeks. A longitudinal study of the cheek-to-cheek diameter is currently in progress.

In conclusion, when precise gestational age is known, cheek-to-cheek diameter measurements can help in the follow-up of fetal growth. In cases with less than certain gestational age, cheek-to-cheek diameter is less useful, but a normal cheek-to-cheek diameter/biparietal diameter ratio is a reassuring index of adequate growth.

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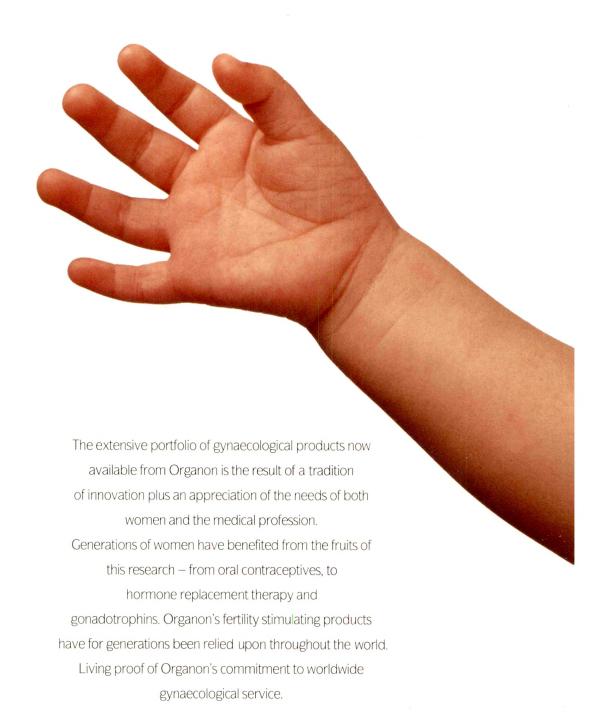
- 1 oral contraceptives
- 2 hormone replacement therapy
- 3 gynaecological disorders
- 4 obstetrics
- 5 fertility stimulation
- 6 diagnostics

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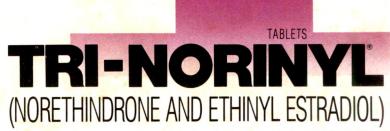
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Serious as well as minor side effects have been reported following the use of oral contraceptives. These include thromboembolic disease.

Please see adjacent page for brief summary of prescribing information.

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ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINY, 21-DAY Tablets provide a notal contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI * **TRI **** **TRI *** **TRI **** **TRI *** **TRI **** **TRI *** **TRI **** *

TRI-MORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 5 yellow green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethrodrom 6.5 mg and Hermy estradiol 0.035 mg, each yellow green tablet contains norethrodrome 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions. Thrombophleibits or thromboembolic disorders • A past history of deep vein thrombophleibits or
thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected
carcinema of the breast • Carcinema of the endometrium, and known or suspected
estrogendependent neoplasus • Undiagnosed abnormal genital bleeding • Cholestatic, jourdice of pregnancy or joundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors •
Known or suspected pregnancy

WARNINGS.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side organics showing increases our list of services carnovascular size effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperipidemias, hypercholes terolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estro-gen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Proughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure case control studies and prospective of conord studies. Lose control studies provide a measure of the relative risk of a disease. Relative risk, the ratio of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Chort studies provide not a measure of the relative risk but a measure of attributable risk, which is the difference in the incidence of disease between eral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

1. Introducemental biodiscretain and other moderate interest and other moderate.

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery discases such as hypertension, hypercholestenlering, montiod obestly and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular discase goals in our women ownens who take and nontraceptives.

is a large production and age of so, inweets, like its like possibility of a list of anounascular disease even in very young women who take oral contraceptives. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the maintity of excess cases

Mortality rates associated with circulatory disease have been shown to increase substantially rs over the age of 35 and non-smokers over the age of 40 among women who use oral

Oral contraceptives may compound the effects of well-known risk factors for coronary artery one contraceptives may compound the effects of wear-anominst factors or commany areity diseases, such as hyperfension, disbets, hyperfindings, hypercholemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose oblerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see WARNINGS, section 9). Similar effects on risk factors have been associated with an increased risk of heard disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

h. Thromboambolism

b. Thromboembolism
An increased risk of thromboembolic and thrombotic disease associated with the use of oral
contraceptives is well established. Case control studies have found the relative risk of users
compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 10
for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for owner with prefision
conditions for venous thromboembolic disease. One cohort study has shown the relative risk to
be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis
or vancose venish and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears
after nell use is chosed. after pill use is stopped.

arter jun user is suppeu.

A2 to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombot) An increase in born the relative and attributable risks of ceretrovascular events (thromobolic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), Inypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhapic strokes.

interactive to increase the risk in humanizing, access and in a large study, the relative risk of thomobic strokes has been shown to range from 3 for nor indensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who used oral contraceptives, 1.8 for nor smokers who used oral contraceptives, 1.8 for nor

who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with swere hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives. A postive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (RDU) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase RDU cholesteroit, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptive. The amount of both hormones should be considered in the choice of a neal contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of

therapeutics. For any particular estrogen/progestogen combination, the dosage regimen pre-scribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

e. Persistence of risk of vascular disease There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for al least 9 years for women 40–49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Birtain, the risk of developing cerebrovascular disease persisted for al least 6 years after discontinuation of oral contrace-ptives, although excess risk was very small. Subaractinoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

were performed wind under contracteptive unautous containing upon in gind in right or estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits. pregnancy in the event of method failure. Each method of contraception has its specific been-fits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smake and 40 and older who do not smake, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1975s—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower settingen does formulations combined with careful in startchon of oral contra-ceptive use to women who do not have the various risk factors listed in this labeling.

ceptive use to women who do not have the various risk hadros istein it his isbeling. Because of these changes in practice and, also, because of some limited new data which sug-gest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1999. The Committee concluded that although cardiovascular dis-ease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical proc-dures which may be necessary if such women do not have access to effective and acceptable means of contraception. means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS
Numerous epidemiological studies have been performed on the incidence of breast, endome
trial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the liter ature suggests that use of oral contraceptives is not associated with an increase in the risk of dure suggests and use or one contractives as the age and parity of first use or with most of the mar-keted brands and doses. The Cancer and Sterrid Hormone study also showed no latent effect on the risk of breast cancer for all teast a decade following long-term use. A first subties have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there con-tinues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervi-cal cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocel-lular carcinoma in long-term (> 8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral con-Traceptives. Oral contraceptives should be discontinued if there is unexplained partial or com-plete loss of vision; onset of proplosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

b. Onat. Continue the continue of the property of the continue that is received in the continue that is the con

concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

J. BALLBLADDER DISEASE Earlier studies have reported an increased lifetime relative risk of galibladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the rela-tive risk of developing galibladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

8. CARBONYDRATE AND LIPID METABOLIC EFFECTS
Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed ear-lier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The inci-dence of risk also was eported to increase with continued use and among older women. Data from the Rayal Cellege of General Practitioners and subsequent randomized trais have shown that the incidence of hypertension increases with increasing concentrations of progestogens. Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contrace-tives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADMANE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on a tives, especially during the first 3 months of use. Non-hormonal causes should b and adequate diagnostic measures taken to rule out malignancy or pregnancy in breakthrough bleeding, as in the case of any abnormal vaginal bleeding, if pathol excluded, there or change to another formulation may solve the problem. In the e orthea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especiall a condition was pre-existent.

PRECAUTIONS 1. PHYSICAL EXAMINATION AND FOLLOW-UP

 PHI STOCKE Comment for A map to Policiary
 A complete medical history and physical examination should be taken prior to the
 reinstitution of oral contraceptives and at least annually during use of oral c
 These physical examinations should include special reference to blood pressure, t
 men and pelvic organs, including cervical cytology and relevant laboratory test undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate dia unes should be conducted to rule out malignancy. Whomen with a strong family his cancer or who have breast nodules should be monitored with particular care.

2 LIPIN NISARNERS

2. LIPPO DISTRUCKS.
Women who are being treated for hyperlipidemias should be followed closely if the oral contraceptives. Some progestogens may elevate LDL levels and may render I hyperlipidemias more difficult.

3. LIVER FUNCTION

It jaundice develops in any woman receiving oral contraceptives the medicati discontinued. Steroid hormones may be poorly metabolized in patients w

Oral contraceptives may cause some degree of fluid retention. They should be pri-caution, and only with careful monitoring, in patients with conditions which mi

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug di depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerand assessed by an ophthalmologist.

DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menst tes have been associated with concomitant use of rifampin. A similar associatio marked, has been suggested with habiturates, phenylbutazone, phenytoin sodiu bly with griseofulvim, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be afficontraceptives:

Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin

norepinephrine-induced platelet aggregability
b. Increased thyroid binding globulin (TBG) leading to increased circulating tota
mone, as measured by protein-bound iodine (PBI), 14 by column or by radiominur
13 resin uptake is decreased, reflecting the elevated TBG. Free 14 concentration c. Other binding proteins may be elevated in serum

d. Sex steroid binding globulins are increased and result in elevated levels of tot sex steroids and corticoids; however, free or biologically active levels remain unc

Set introductions in the continuous interests and the continuous and t gnificance if a woman becomes pregnant shortly after discontinuing oral contri

10. PREGNANCY

Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of ers and a few adverse effects on the child have been reported, including jaundic enlargement. In addition, oral contraceptives given in the postpartum period may i lactation by decreasing the quantity and quality of breast milk. If possible, the nu should be advised not to use oral contraceptives but to use other forms of contra she has completely weaned her child. INFORMATION FOR THE PATIENT See PATIENT LABELING.

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated of eral contraceptives (see **WARNINGS** section): Infrombophilebitis - Arterial t isim - Pulmonary embolism - Myocardial infarction - Cerebral hemorrhage - Ceresis - Hypertension - Galibladder disease - Mepatic adenomas, caraniomas or

There is evidence of an association between the following conditions and the use ϵ ceptives, although additional confirmatory studies are needed: Mesenteric thron nal thrombosis

nat thromosiss. The following adverse reactions have been reported in patients receiving oral or and are believed to be drug-related. Nausea * Vornting * Gastromtestinal symptic abdominal cramps and bloating) * Breakthrough bleeding * Spotting * Change in me. Amenorrhea * Temporary infertility after discontinuation of treatment * Edem. which may persist * Breast changes: tenderness, enlargement, secretion * Change incervical erosion and secretion * Disminution when given immediately postpartum * Cholestatic jaundice * Migraine * Rash (all tal depression * Reduced tiderance to carbohydrates * Vaginal candidiasis * Chan curvature (steepening) * Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contracept association has been neither confirmed nor refuted. Pre-menstrual syndrome - Changes in appetite - Cystiths-like syndrome - Headache - Nervousness - Dizzm ism - Loss of Skalp hair - Erythema multiforme - Erythema nodosum - Hemorrhag Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Bud ime • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large dose: traceptives by young children. Overdosage may cause nausea, and withdrawal b occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contra supported by epidemiological studies which largely utilized oral contraceptive containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of Effects on menses: • Increased menstrual cycle regularity • Decreased bloc decreased incidence of iron deficiency anemia • Decreased incidence of dysmeno Effects related to Inhibition of ovulation: • Decreased incidence of functional ova

Decreased incidence of ectipic pregnancies

Effects from long-term use:

Decreased incidence of fibroadenomas and fibrocy
of the breast

Decreased incidence of acute pelvic inflammatory disease

Decreased incidence of acute pelvic inflammatory disease of acute pelvic inflammatory disease of acute pelvic dence of endometrial cancer . Decreased incidence of ovarian cancer

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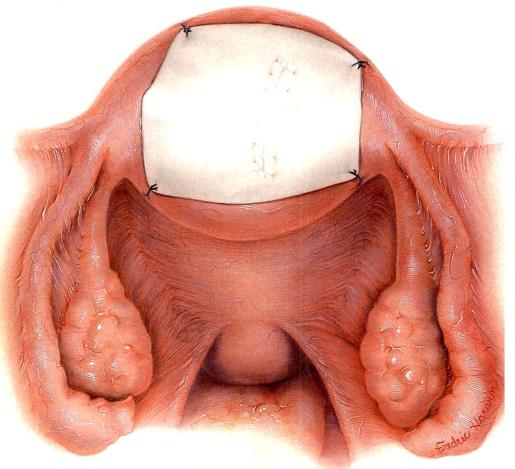
ence between life nan and her unborn ow and give copies ts, even if you don't roblem. Even if they t. Because if your now the right the time and

Drugs and Pregnancy: Did you know?

- **1.** Did you know that if you use cocaine while you're pregnant, you have a much greater chance of having a miscarriage?
- **2.** Did you know that if you use cocaine, you can pass it on to your baby through your breast milk?
- **3.** Did you know that if you use any illicit drug while you're pregnant, there's a greater chance that you will have a low-birthweight baby?
- **4.** Did you know that low birthweight is associated with a greater risk of infant death and mental and physical handicaps?
- **5.** Did you know that heroin use during pregnancy increases the likelihood of stillbirth and death among newborns?
- **6.** Did you know that if you or any of your sexual partners ever used an illicit IV drug, you may be infected with the AIDS virus (HIV), and that you can pass that virus on to your unborn child?
- **7.** Did you know that if you use cocaine, are addicted to heroin, or are methadone-dependent, your children will be at a greater risk for sudden infant death syndrome (SIDS)?
- **8.** Did you know that if you use barbiturates during your pregnancy you can change your child's normal patterns of growth and behavior? Your child might also suffer withdrawal symptoms for months after birth, including high-pitched crying, irritability, and sleep disturbances.
- **9.** Is your doctor aware of all the prescription and/or illicit drugs you may be taking?

Did you know that if you have a drug problem, there are many people and resources you can turn to for help? Just ask your doctor.

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n Peritoneal

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Convenient

Available in specific configurations tailored for peritoneal reconstruction; the GORE-TEX Surgical Membrane — *Configured for Peritoneal Reconstructic* is packaged sterile and **can be resterilized**.

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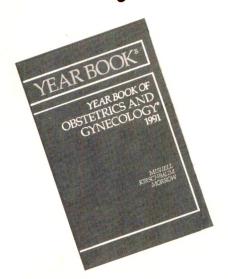
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 Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.
 See prescribing information.

See brief summary on adjacent page.

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riphasil Levonorgestrel and ethinyl estradiol tablets-

THE OC TO START WITH BECAUSE SHE'LL STAY WITH IT

IN BRIEF:
TRIPHASIL.** — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol; 7 light-green tablets containing inert ingredients are included in the 28-day regimen! — Triphasic regimen.

Indications and Usage — TRIPHASIL.** is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (0Cs) as a method of contraception.

Contraindications — OCs should not be used in women with any of the following: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 2. A past history of deep-vein thrombophlebits or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Endometrial carcinoma or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contra-ceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

ceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

Use of CCs is associated with increased risks of serious conditions including myocardial infarction, thrombomboism, stroke, hepatic neoplasia, galibladder disease, and hypertension, although risk of serious morbidity/mortality is very small in healthy women without underlying risk factors. Morbidity/mortality risk increases significantly if other risk factors present (i.e. hypertension, hyperipidemias, besity, diabetes). Practitioners prescribing CCs should be familiar with the following information relating to these risks. This information is based principally on data involving OCs with higher doses of estrogen and progestogen than those commonly used today. Effect of long-term use of lower estrogen and progestogen formulations is yet to be determined.)

1. Thromboemboiic Disorders and Other Vascular Problems — MYOCARDIAL INFARCTION (MI). An increased risk of MI has been attributed to OC use. Risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease (i.e. hypertension, hypercholesterolemia, morbid obesity, diabetes). Relative risk of heard attack for current OC users is estimated to be two to six, risk is very low under the age of 30. Smoking combined with OC use contributes substantially to incidence of MIs in women in their mid-thirties or older with smoking accounting for majority of excess cases. Mortality rates associated with circulatory disease increase substantially in smokers over the age of 30 and nonsmokers over the age of 40 among OC users.

OCs may compound effects of well-known risk factors such as hypertension, diabetes, hypertipidemias, age and obesity, in particular, some progestogens decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperisualinism. OCs have been shown to increase brook

The dosage regimen prescribed should contain the least amount of estrogen and progestogen compatible with a low failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of

iow failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of estrogen.

PERSISTENCE OF RISK OF VASCULAR DISEASE. Two studies have shown persistence of vascular disease risk for ever-users of OCs. In a U.S. study, MI risk after OC discontinuation persists for at least 9 years in women 40-49 years who had used OCs for five or more years; increased risk was not demonstrated in other age groups. In a study in Creat Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after OCs stopped, although excess risk was very small. Both studies used OC formulations with 50 micrograms or higher of estrogens.

of estrogens.

2. Estimates of Mortality from Contraceptive Use — A study using data from several sources concluded that with the exception of OC users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The possibility of increased mortality isk with age for OC users is based on data from the 1970s — but reported in 1983. However, current practice involves use of lower estrogen dose formulations combined with careful restriction of OC use to women without the various risk factors listed in this labeling. Changes in practice and new data suggesting that cardiovascular disease risk with OCs may be less than previously observed prompted the Fertility and Maternal Health Drugs Advisory Committee to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with OC use after age 40 in healthy nonsmokers leven with newer low-dose formulations), greater potential health risks are associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if effective, acceptable contraception is not available.

The Committee concluded that the benefits of OC use by healthy nonsmoking women over 40 may outweigh the possible risks. Older women, as all women who take OCs, should use the lowest possible effective dose formulations.

formulation.

3. Carcinoma of the Reproductive Organs — Numerous epidemiological studies have looked at the incidence of breast, endometrial, ovarian and cervical cancer in women using OCs. Overwhelming evidence suggests that OC use is not associated with an increase in risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. A few studies show a slightly increased relative risk of developing breast cancer, although the methodology of these studies, including differences in examination of users and nonusers, and in age at start of use, has been questioned.

Some studies suggest that OC use is associated with an increased risk of cervical intraepithelial neoplasia in some populations of women. However, controversy continues about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between OC use and breast and cervical cancers, a cause and effect relationship has not been established.

A Hepatic Neoplasia — Benign hepatic adenomas are associated with OC use, although incidence is rare in the U.S. Indirect calculations estimate attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

British studies have shown an increased risk of hepatocellular carcinoma in long-term (> 8 years) OC users, these cancers are extremely rare in the U.S. and attributable risk (excess incidence) of liver cancers in OC users approaches less than one per million users.

5. Ocular Lesions — There are clinical case reports of retinal thrombosis with OC use. Discontinue OCs if there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions; undertake appropriate diagnostic and therapeutic measures immediately.

lesions: undertake appropriate diagnostic and therapeutic measures immediately
6. Oral-Contraceptive Use Before or During Early Pregnancy — Extensive epidemiological studies revealed no
increased risk of birth defects when OCs used prior to pregnancy. Studies do not suggest a teratogenic effect,
particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently
during early pregnancy. OC induced withdrawal bleeding should not be used as a pregnancy test Do not use
OCs during pregnancy to treat threatened or habitual abortion. Rule out pregnancy if two consecutive periods
missed before continuing OC use. If patient has not adhered to prescribed schedule, consider pregnancy at time
of first missed period. Discontinue OC if pregnancy confirmed.
7. Gallbladder Disease — Earlier studies reported an increased lifetime relative risk of gallbladder surgery in users
of OCs and estrogens, more recent studies show that the relative risk of developing galibladder disease among
OC users may be minimal, which may be related to use of formulations with lower hormonal estrogen and
progestogen doses.

progestogen doses.

progestogen doses.

8. Carbohydrate and Lipid Metabolic Effects — OCs cause glucose intolerance in a significant percentage of users. OCs with greater than 75 µg of estrogen cause hyperinsulinism; lower estrogen doses cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance; leffect varies with different agents). Observe prediabetic and diabetic women carefully while taking OCs. In non-diabetic women, OCs have no apparent effect on fasting blood glucose.

8. small proportion of women will have persistent hypertriglyceridenia while on OCs. Changes in serum triglycerides and lipoprotein levels have been reported in OC users (see Warnings).

9. Elevated Blood Pressure — Increase in blood pressure has been reported in women on OCs; increase is more likely in older OC users and with continued use. Data show that incidence of hypertension increases with increasing quantities of progestogens.

Ecourade women with history of hypertension or hypertension-related diseases, or renal disease to use another.

increasing quantities of progestogens.

Encourage women with history of hypertension or hypertension-related diseases, or read disease to use another contraceptive method. Monitor hypertensive women electing to use OSc closely, discontinue OC if significant blood pressure elevation occurs. For most women, elevated blood pressure returns to normal after OC stopped. No difference in occurrence of hypertension among ever- and never-users exists.

10. Heardace—Discontinue OC and evaluate cause at onset or exacerbation of migraine, or if new pattern of headache (i.e. recurrent, persistent, severe) develops.

11. Bleeding Irregularities—Breakthrough bleeding and spotting sometimes occur especially during first 3 months of use. Type and dose of progestogen may be important. Consider non-hormonal causes and take adequate diagnostic measures to rule out malignancy or pregnancy in event of breakthrough bleeding, as with any abnormal vaginal bleeding. If pathology excluded, time or a formulation change may solve the problem. In the event of amenorrhea, rule out pregnancy. Some women encounter post-pill amenorrhea or oligomenorrhea.

Precautions

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event of aniethoriteat, this out pregnancy. Some women encounter post-pili amenormea of oligomenormea, especially when such a condition was pre-existent.

Precautions

1. Physical Examination and Follow Up.—A complete medical history and physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical examination should be special reference to blood pressure. Deress a adorner and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, conduct appropriate diagnostic measures to rule out malignancy. Monitor women with strong family history of breast cancer or who have breast notules with particular care. 2. Liquid Disorders.—Follow women being treated for hyperipidemias closely if they elect to use CCs. Some progestogens may elevate LDL levels and may render control of hyperipidemias more difficult. See Warningsi 3. Liver Function.—Discontinue OC it jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. Fluid Retention — OCs may cause some degree of fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions possibly aggivared by fluid retention. 5. Emotional Disorders.—It significant depression occurs stop medication and use alternate contraceptive method in attempts to determine if symptom is drug related. Observe carefully those with history of depression and stop drug if depression necurs to serious degree. 6. Contact. Lenses—Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. Drug Interactions—Reduced efficacy and increased increace of breakthrough bleeding and menstrual irregulanties are associated with concomitant rifampia use. A similar association, though less marked. Is suggeste

compretely wearned

Information for the Patient — See Patient Package Labeling.

Adverse Reactions — An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis; arterial thromboembolism; pulmonary embolism; myocardial infarction; cerebral hemorrhage; cerebral thrombosis, hypertension; gallibladder disease; hepatic adenomas or benign

There is evidence of an association between the following conditions and OC use, although additiona confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis.

confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis.

The following adverse reactions have been reported in patients on OCs and are believed to be drug-related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenormea; temporary infertifity after treatment discontinued; edema; melasma which may persist breast changes: tenderness, enlargement; secretion; change in weight fincrease or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice, migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in OC users and the association is neither confirmed nor refuterd congenital anomalies; orgenestical syndrome; catagets, ordine querifis changes in apostite, custific like.

The following adverse reactions have been reported in OC users and the association is neither confirmed nor refuted: congenital anomalies; premenstrual syndrome; cataracts, optic neuritis; changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsuitism; loss of scalp hate, erythema multiforme; entrema nodosum; hemorrhagic eruption; vaginitis; porphyria; impaired renal function; hemolytic uremic syndrome; budd-Chiari syndrome, actre; changes in libido; colitis, sickle-cell disease; cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes.

Dverdosage—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits—The following noncontraceptive health benefits related to OC use are supported by epidemiological studies that largely utilized OC formulations containing doses exceeding 0.035 mg of thinly distradiol or 0.05 mg of mestranol. **Effects or mensural cybic regularity, bedereased blood loss and decreased incidence of iron-deficiency anemia; decreased incidence of dysmenorma. **Effects related to inhibition of ovulation;* decreased incidence of tectopic pregnancies. **Effects from long-term use: decreased incidence of the preast; decreased incidence of acute pelvic inflammatory disease; decreased incidence of endometrial cancer; decreased incidence of acute pelvic inflammatory disease; decreased incidence of endometrial cancer; decreased incidence of acute pelvic inflammatory disease; decreased incidence of endometrial cancer; decreased and **Administration**—For maximum contraceptive effectiveness, take TRIPHASIL** (levonorgestrel

Dosage and Administration — For maximum contraceptive effectiveness, take TRIPHASIL* (levonorgestrel and ethinyl estradiol tablets — triphasic regimen 21- and 28-day regimens) exactly as directed and at intervals not over 24 hours.

not over 24 flutus. (If TRIPHASI): 's first taken later than first day of first menstrual cycle of medication or postpartum, contra-ceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.) For full details on dosage and administration see prescribing information in package insert.

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Transforming growth factor- β_1 expression during placental development

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Placental growth has several malignant characteristics, including properties of invasiveness, rapid cell proliferation, and a lack of cell contact inhibition. These malignant characteristics of placental development are strictly regulated throughout normal gestation, because placental growth is limited in both extent and duration. Transforming growth factor- β_1 inhibits growth of many normal and malignant cell lines. In this study, using Northern blot analysis, we found transforming growth factor- β_1 expression to occur in human placenta throughout gestation. Peak expression was noted at midgestation (near 17 weeks) and again in late gestation (near 34 weeks). Immunohistochemical analysis localized transforming growth factor- β_1 protein expression to the syncytiotrophoblastic layer. The process of trophoblastic invasion of the decidua and myometrium is usually complete by 18 weeks of gestation, and absolute growth of the placenta ceases in late gestation (near 35 weeks). The time frames of maximal transforming growth factor- β_1 expression noted in our studies correlate with these events. We speculate that peak transforming growth factor- β_1 expression at these stages of placental development is suggestive of its regulation of both trophoblastic invasion and proliferation. (AM J OBSTET GYNECOL 1991;165:853-7.)

Key words: Transforming growth factor- β_1 , human placenta

Placental implantation and subsequent growth and development have been described as a pseudomalignant process. Similarities between trophoblastic cell growth and malignant cells include invasiveness, rapid cell proliferation, lack of cell contact inhibition, and a degree of immune privilege. These malignant characteristics of placental development appear to be strictly regulated throughout normal gestation, because placental growth is limited in both its extent and duration. In fact, during embryogenesis and placentation tissues and organs develop in a sequential pattern, implying that genes coding for particular proteins are turned on and off in a precise and well-regulated manner. It is now becoming apparent that growth inhibitors play an important role in maintaining tissue homeostasis. Peptide growth factors, active in common regulatory pathways, may be important regulators of placental development.

Transforming growth factor- β_1 (TGF- β_1) is a poly-

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peptide originally identified in tumor cells and postulated to be involved in transformation and neoplastic growth. Its presence in numerous tissues,1-4 however, implies a normal physiologic role that appears to be multifunctional. TGF- β_1 has been shown to stimulate growth of some cell types but to inhibit growth of others, to stimulate growth of fibroblasts and promote wound healing,5.6 and to induce differentiation of leukemic and bronchial epithelial cell lines.7.8 A fundamental property of TGF-β₁ is its ability to inhibit proliferation of both normal and malignant cell lines. TGF- β_1 has been shown to inhibit growth of different ovarian cancer cell lines, as well as some human leukemic cell lines.^{8,9} As such, we hypothesized that TGF-β₁ may play an important role in the inhibition of trophoblastic invasion of the myometrium during placental development. In humans, this process is largely limited to the eighth week through the eighteenth week of gestation.10 We evaluated the temporal expression of TGF- β_1 in human placenta to begin to examine this hypothesis.

Material and methods

Collection of placental tissue. Placental tissue was obtained after curettage, spontaneous delivery, or cesarean section and immediately placed in liquid nitrogen. Informed consent was obtained from patients in all instances, in accordance with the protocol approved by our institutional review board. Time frames of placental tissue studied were chosen to correlate with early, mid, and late gestation. Placental tissue obtained from a total of 20 patients was used for analysis. Initially, a

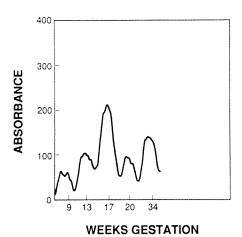


Fig. 1. Graph showing results of scanning densitometry of blots of five representative RNA samples. TGF- β_1 expression is noted throughout gestational period shown, with peak expression at 17 and 34 weeks' gestation.

panel of total ribonucleic acid (RNA) from placental tissues ranging from 6 to 40 weeks' gestation was studied with Northern blot analysis. These studies were repeated with total RNA from different placental tissues of similar gestational ages. Three panels of RNA were studied. Additionally, Northern blot analysis was performed with 100 µg of total RNA from placental tissue of 15 through 19 weeks' gestational age and 32 through 34 weeks' gestational age to further evaluate time frames where peak expression had previously been identified.

RNA isolation. Total RNA was isolated from the placental tissues according to the technique of Chomzynski and Sacchi.11 Briefly, approximately 2 gm of placental tissue was homogenized in 10 ml extraction solution (4 mol/L guanidine thiocyanate; 25 mmol/L sodium citrate, pH 7.0; 0.5% sarcosyl; 0.1 mol/L 2-β-mercaptoethanol; 2 mol/L sodium acetate; and 10 ml phenol). The homogenate was mixed with chloroform/isoamyl alcohol (2 ml of 49:1 solution) and chilled on ice for 15 minutes. After centrifugation for 20 minutes at 10,000 g at 4° C, the aqueous phase was removed and precipitated with one volume of isopropanol at -20° C for 2 hours. This was followed by a second centrifugation for 20 minutes at 10,000 g at 4° C. The resultant pellet was dissolved in extraction solution (600 μl) and precipitated with isopropanol (1:1, vol/vol) and sodium acetate (1:10, vol/vol) at -20° C for approximately 2 hours. After centrifugation for 10 minutes at 4° C, the pellet was washed with 70% ethanol and vacuum dried for approximately 5 minutes. The pellet was then resuspended in diethylprocarbonate-treated water. Total RNA concentration was quantitated by absorption spectrophotometry at 260 nm.

Northern blot analysis. RNA samples (100 µg) were

resolved by electrophoresis on formaldehyde agarose gels and transferred to nitrocellulose membranes by towel blotting. The integrity and quantity of each RNA sample were ensured by staining the gel with ethidium bromide and visualizing the gel under ultraviolet transillumination. The filters were prehybridized in hybridization solution containing 10× Denhardt's solution, 0.1% sodium dodedecyl sulfate, 100 µg/ml single standard sperm deoxyribonucleic acid, 50% deionized formamide, and a $5 \times$ solution of 3 mol/L sodium chloride, 0.1 mol/L sodium phosphate, 0.02 mol/L ethylenediaminetetraacetic acid, and sodium hydroxide and then hybridized in the same solution containing a phosphorus 32-labeled human TGF-β₁ complementary deoxyribonucleic acid probe (1 \times 10% counts/min/ml) at 42° C overnight. After four washings (final wash 1× saline sodium citrate buffer with 0.1% sodium dodedecyl sulfate at 55° C for 20 minutes) the filters were subjected to autoradiography. The position of 18S and 28S RNA was determined, and the single band observed, which corresponded to the position of TGF-β₁ messenger RNA (mRNA) (2.1 kb), was quantitated by scanning densitometry with a Hoefer GS 300 (Hoefer Scientific Instruments, San Francisco) gel

Our initial studies with 25 and 50 μg of total RNA revealed expression only at 17 and 34 weeks' gestation. In later blots TGF- β_1 expression was noted at all time points when 100 μg of total RNA was used. For this reason 100 μg samples were used in all experiments reported in this study.

Immunohistochemical analysis. Placental sections (10 µm) from 17 and 34 weeks' gestation were fixed in chilled methanol, followed by fixation in sodium periodate, lysine, and paraformaldehyde solution. The sections were then incubated at room temperature with a phosphate-buffered saline solution containing bovine serum albumin (1%) and 0.1% Triton-X-100 (Fischer Scientific, Fair Lawn, N.J.) compound for 10 minutes, followed by a 30-minute incubation with phosphatebuffered saline solution containing 0.2% gelatin to minimize background contamination. The sections were then incubated with polyclonal rabbit antibody against human TGF-B₁ (1:50 dilution in phosphate-buffered saline solution-bovine serum albumin) for 1 hour at 37° C. TGF-β₁ antibody used in this study was obtained from Collagen Corporation, Palo Alto, Calif. After three washings with phosphate-buffered saline solution containing 0.2% gelatin for 15 minutes each, the specimens were incubated with fluorescein-conjugated goat anti-rabbit polyclonal antibody (1:50 dilution in phosphate-buffered saline solution—bovine serum albumin) for 45 minutes at 37° C. After three washings in phosphate-buffered saline solution containing 0.2% gelatin, immunofluorescent sites were visualized in an epiflu-

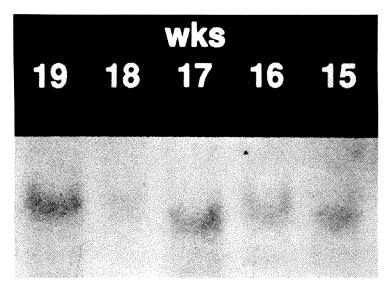


Fig. 2. Northern blot of total RNA samples isolated from placental tissues near midgestation. TGF- β_1 expression is noted throughout (15 to 19 weeks). Maximal expression was found at 17 weeks' gestation by densitometric quantitation.

orescence microscope (Leitz, Wetzler, Germany). Sequential sections were treated in a similar manner with nonimmune rabbit serum substituted at the same concentration for TGF- β_1 antibody to serve as control.

Results

With Northern blot analysis and 100 µg of total RNA, we were able to identify TGF-β, mRNA expression throughout the gestational ages studied (6 through 40 weeks). Fig. 1 represents the continuous scanning densitometry graph of a blot of equal amounts of total RNA isolated from placental tissue ranging from 9 to 34 weeks' gestation. Expression of TGF-β, was noted throughout the time points examined, with peaks of expression noted at 17 and 34 weeks' gestation. This is consistent with our initial findings of TGF-β₁ mRNA expression only at 17 and 34 weeks' gestation using lower amounts of total RNA (25 and 50 μg).

Northern blot analysis of total RNA isolated from tissue samples ranging from 15 to 19 weeks' gestation revealed TGF-β, expression throughout these time points (Fig. 2). Maximal expression was observed at 17 weeks by densitometric quantitation. Analysis of total RNA isolated from tissue of 32 to 34 weeks' gestation revealed minimal expression in 32 and 33 week samples with a striking increase of expression occurring at 34 weeks (Fig. 3).

We have looked at the expression of c-jun and jun-B in human placenta. These protooncogenes have been associated with cellular growth processes, including both proliferation and differentiation. Their patterns of expression did not correspond to that found with TGF- β_1 (unpublished data). This would imply that the

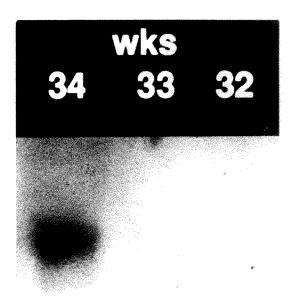


Fig. 3. Northern blot of total RNA samples of placental tissue in late gestation. Minimal expression occurred at 32 and 33 weeks with increased expression at 34 weeks.

increased expression of TGF-β₁ near 17 and 34 weeks' gestation is not related to a general phenomenon.

After identification of peak TGF-β, mRNA expression in 17 and 34 weeks' gestational age placental tissue, we used immunohistochemical analysis to localize TGF- β_1 protein expression in these tissues. TGF- β_1 protein expression was localized to the periphery of placental villi corresponding with the syncytiotrophoblastic layer (Fig. 4). There was an absence of staining of cell types of the villous core. Control specimens did not show this pattern of staining.



Fig. 4. Immunohistochemical analysis of 17-week placental tissue. TGF-β₁ protein expression is localized to syncytiotrophoblastic layer only. (Original magnification ×100.)

Comment

The placenta is a very dynamic organ, passing through stages of graft acceptance, cell differentiation, invasive growth, and cessation of both growth and invasiveness. Knowledge about regulatory factors involved in these processes is limited. TGF-β₁ promotes proliferation of some cell types but inhibits proliferation of others, depending on cell conditions and concentration. A fundamental property is its ability to inhibit proliferation of both normal and malignant cell lines.

The observations made in our study suggest that TGF-β₁ is expressed in human placenta throughout gestation. Additionally, two peaks of expression were noted: an initial peak occurring near midgestation at 17 weeks and a second peak in later gestation, near 34 weeks. In humans trophoblastic invasion of the myometrium is largely limited to the eighth through eighteenth weeks of gestation. Large numbers of cytotrophoblastic cells invade the decidua basalis and underlying myometrium. Fusion of cytotrophoblastic cells results in formation of syncytial cells. An absence of mitotic activity is noted in the syncytiotrophoblast. 12 Increased mRNA expression of TGF-β₁ near 17 weeks' gestation suggests that it may play a role in inhibition of myometrial trophoblastic invasion. Also, TGF-β₁ protein expression was localized to the syncytiotrophoblastic layer with immunohistochemical analysis.

The expression of TGF-β₁, a factor known to be related to inhibition of cell proliferation, in these mitotic endstage cells supports its possible role in the inhibition of cytotrophoblastic myometrial invasion. It is possible that different trophoblastic cell types may express TGF- β_1 near midgestation and be involved in myometrial invasion. Our studies, however, were able to identify only TGF-β, protein expression localized to the villous syncytiotrophoblast with an absence of staining of other cell types. Although we cannot rule out decidual contamination as a source of TGF-β₁ mRNA expression in our specimens, immunohistochemical analysis identified TGF-β, protein expression only in the syncytiotrophoblastic layer.

Absolute growth of human placenta as determined by deoxyribonucleic acid content declines in late gestation beginning at 35 to 36 weeks' gestation, at which time cytotrophoblastic proliferation essentially ceases.¹³ TGF-β₁ has been shown to inhibit rat trophoblastic cell deoxyribonucleic acid synthesis in a dose-dependent manner.¹⁴ A second peak of TGF-β₁ expression near 34 weeks' gestation may therefore be related to this cessation of cytotrophoblastic cell proliferation and decline in placental growth. This was again supported by localization of TGF-B1 protein expression in the syncytiotrophoblastic layer with immunohistochemical analysis.

In summary, with Northern blot analysis TGF-β,

expression was noted throughout gestation in our study. Peak expression was noted at 17 and 34 weeks' gestation. Immunohistochemical staining revealed TGF-β₁ expression restricted to the syncytiotrophoblastic layer. TGF-β₁ expression in the syncytiotrophoblast at these time points may be related to inhibition of cytotrophoblastic proliferation occurring at these stages of placental development. Pathologic conditions of trophoblastic invasion exhibited in gestational trophoblastic disease and choriocarcinoma represent a deviation from normal trophoblastic growth. We have not examined such tissues. Examination of these tissues with comparison to findings in nonpathologic conditions would be of value in further elucidation of the role of TGF-β₁ in regulating trophoblastic myometrial invasion.

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Multicenter randomized clinical trial of home uterine activity monitoring for detection of preterm labor

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Home uterine activity monitoring has been described as an effective means of detecting uterine contractions, but controversy exists whether it is home uterine activity monitoring or increased nursing support in conjunction with it that contributes to earlier detection of preterm labor. In this study 377 women at risk for preterm labor from three centers were prospectively, randomly assigned to high-risk prenatal care alone (not monitored) or to the same care with twice-daily home uterine activity monitoring without increased nursing support (monitored). The two groups were medically and demographically similar at entry into the study. Routine visits, nonroutine visits, and gestational age at diagnosis of preterm labor were similar in both groups. Preterm labor occurred in 41 of 198 monitored and 39 of 179 not monitored patients. Mean cervical dilatation was 1.4 cm in 41 monitored compared with 2.5 cm for 37 not monitored ($\rho = 0.0006$); 73.1% of monitored and 27.5% of not monitored had preterm labor detected before 2 cm dilatation (p = 0.00009). Neonatal outcome of singleton pregnancies showed greater birth weight, fewer days in the neonatal intensive care unit, and fewer babies requiring oxygen therapy and mechanical ventilation in the monitored group. The better outcomes are probably due to the increased likelihood of diagnosis of preterm labor before advanced cervical dilatation with home uterine activity monitoring, thus providing the clinician with a better chance to initiate tocolytic therapy directed at improving pregnancy outcome. (AM J OBSTET GYNECOL 1991;165:858-66.)

Key words: Home uterine activity monitoring, preterm labor

Preterm delivery is the most significant contributor to perinatal morbidity and mortality in the United States.1 Unfortunately, many episodes of preterm labor are not detected until it is too late to provide effective tocolytic therapy. Recent studies have shown that an increase in the number of uterine contractions precedes the onset of preterm labor and is present in early labor; however, women often are not aware of the increased number of contractions until labor is advanced.2.3 Studies showing that devices designed to monitor uterine activity offer a more reliable method of quantitating contractions suggest that home uterine activity monitoring may allow earlier detection of the onset of preterm labor.4,5 Although many studies examining its efficacy have suggested beneficial effects, most of these studies have included intensive perinatal nursing support in combination with the objective data obtained with a device.⁵⁻⁷ Therefore it has not been possible to separate the effects of the detection of increased uterine activity. A prospective, randomized, blinded, multicenter trial of a home uterine activity monitoring device as the sole addition to current standard high-risk obstetric care was conducted to evaluate the effect of objective measurement of uterine activity in the diagnosis of preterm labor.

Methods

Recruitment and eligibility. Prenatal charts at three study sites, Truman Medical Center, Kansas City, Mo., State University of New York Health Science Center, Syracuse, N.Y., and the University of Illinois Hospital, Chicago, were reviewed at the time of the initial prenatal visit, at 22 to 26 weeks' gestation, and at 32 weeks' gestation. Recruitment was conducted from September 1988 to August 1989 at Truman Medical Center and the State University of New York, Syracuse, but was terminated in June 1989 at the University of Illinois because of intended closure of the hospital. Subjects were eligible for recruitment if they were found to have an increased risk of preterm labor as judged by a Creasy risk score of ≥10.8 Women were excluded if they had significant psychiatric problems precluding compliance with the study protocol, did not speak English, or were >32 weeks' gestation. Eligible patients were approached for informed consent and participation in the

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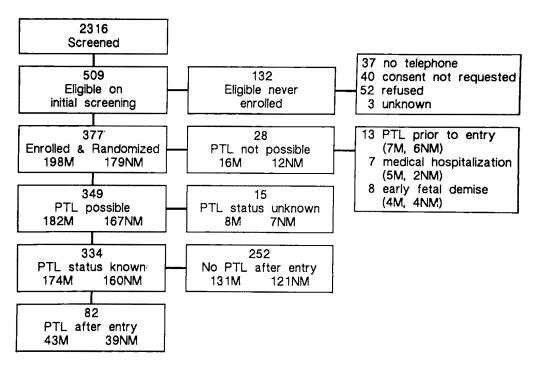


Fig. 1. Study population flow chart. M, Monitored; NM, not monitored; PTL, preterm labor.

study according to a protocol approved by institutional review boards at each study site. The 52 eligible women who refused randomization had Creasy risk scores similar to those of study participants. No other data are available on these women. The flow of patients from 2316 initially screened for eligibility through the 377 who were enrolled and randomized is shown in Fig. 1.

Randomization. All randomization and group assignment was performed by study personnel without direct patient care responsibilities. After enrollment, the group assignments were made by opening consecutively numbered envelopes that randomized patients by a table of random numbers. A different random number sequence was used for each study site, and each site had separate randomization sets for singletons and multiple gestations. No local study personnel had access to the group assignment.

On the basis of these procedures, the 377 patients were assigned to either the monitored group (n = 198) or the not monitored group (n = 179) (Fig. 1). The distribution of monitored and not monitored patients by site was as followed: Truman Medical Center had 82 monitored and 82 not monitored patients; State University of New York had 74 monitored and 68 not monitored patients; and University of Illinois had 42 monitored and 29 not monitored patients. The not monitored group received standard high-risk obstetric care, whereas the monitored group received the same standard high-risk obstetric care plus twice daily home uterine activity monitoring.

Obstetric care

Education. All patients, regardless of group assignment, received education regarding why they were at risk for preterm labor and information about preterm labor precautions, including these signs and symptoms of preterm labor: (1) uterine contractions, (2) dull, low backache, (3) menstrual-like cramps, (4) diarrhea or indigestion, (5) pressure or heaviness in the lower abdomen, back, pelvis, or thighs, (6) vaginal discharge, and (7) vaginal drainage or leaking of amniotic fluid. They were also instructed to notify their physician or clinic if they suspected preterm labor. All patients received education about the monitoring protocol (see below) and uterine self-palpation.

Monitoring protocol. The home uterine activity monitoring device used for this study was the Genesis System (Physiologic Diagnostic Service, Atlanta). The system consists of a guard ring type tocodynamometer connected to a portable battery-operated data recorder, a communication module allowing transmission of data over standard telephone lines, and a receiving computer with strip chart.

Patients in the monitored group received instruction on use of the Genesis System at 24 to 26 weeks or at time of entry into the study if they were enrolled at 26 to 32 weeks. Patients were to monitor for 1 hour twice daily, 7 days per weeks. Monitoring began at 24 weeks and continued to 37 weeks or delivery, if delivery occurred at <37 weeks. If patients entered the study after 24 weeks' gestation, they began monitoring on enroll860 Mou et al.

ment. The patients were scheduled for twice-daily (in the morning and evening) uterine activity data transmission. No advice or instructions were given over the telephone. In addition, patients were instructed to use the system for 1 hour if they experienced any signs or symptoms of preterm labor. These unscheduled sessions were transmitted immediately. All monitoring was performed in the patient's home, and the uterine activity data were transmitted by telephone to a remote site. The receiver personnel served to facilitate the transmission of the uterine contraction data and report the number of contractions to the patient. Patients were previously informed that the personnel would not be able to answer questions, and the personnel were instructed to neither ask medical questions nor give medical advice. The investigators verified on an ongoing basis that the personnel provided no medical information to the monitored patients.

Procedures for routine and nonroutine visits. For both routine and nonroutine care, women in both groups were seen by obstetric caretakers as part of the usual high-risk obstetric care provided at their respective perinatal sites. The minimum care scheduled was a visit every 4 weeks until 30 weeks' gestation, at least every 2 weeks between 30 and 36 weeks, and at least weekly thereafter. The actual schedule was based on each patient's medical problems, and, in general, subjects had more scheduled routine visits than the minimum required.

Nonroutine visits were indicated if the patient had signs or symptoms of preterm labor as described above. Nonroutine visits were obtained at the obstetric care provider's office or clinic during the day, and in hospital labor and delivery units, at night. The indications and procedures followed for nonroutine visits were the same for the two groups. In the case of suspected uterine contractions, the action to be taken by the patient was the same if the monitoring data showed increased contractions or if the patient had the sensation of increased contractions. It consisted of the following: (1) If, on the basis of patient sensation or monitoring, there were four or more contractions per hour lasting >40 seconds, the patient was to assume a left recumbent position, take oral hydration, empty the bladder, and then reassess the number of contractions for 1 hour. (2) If on reassessment there were four or more contractions per hour, then a nonroutine medical visit was indicated. (3) For twin gestations, six contractions per hour rather than four were used in this protocol. This was based on data showing higher baseline contraction frequencies at each week of gestation from 23 to 36 weeks in twin gestations as compared with singleton gestations.5,9 All patients were allowed to arrange a

nonroutine visit for prompt evaluation if they felt it was necessary.

When patients were seen with possible preterm labor, fetal monitoring and cervical examinations were performed by experienced examiners (senior residents or attending physicians) who were not aware of the subject's group assignment. Preterm labor was defined, before the beginning of the study, as four or more contractions per hour plus a change in cervical examination, or four or more contractions per hour and a cervical examination showing >2 cm dilatation and 75% effacement and/or ruptured membranes. In actuality, patients who were diagnosed with preterm labor all had a cervical change of effacement or dilatation as compared with their most recent cervical examination. Tocolytic therapy was prescribed at each of the study sites according to previously existing protocols. In general, treatment at all sites for established preterm labor included bed rest, hydration, evaluation for acute medical problems, and, if no contraindications for drug treatment existed, infusion of one of the common intravenous tocolytics (ritodrine hydrochloride, magnesium sulfate, or terbutaline). If labor stopped, this intravenous therapy was followed by oral tocolytic therapy.

Blinding. All patients were instructed not to inform caretakers of group assignment. Caretakers were informed that they were seeing a study patient but not told the group assignment. If uterine contractions were suspected, caretakers were not informed if the contractions were detected by the monitor or the patient. If a potential examiner inadvertently learned a patient's group assignment, then the study cervical examination was performed by a different experienced caretaker who was not aware of the group assignment.

Statistics

Sample size. As part of planning this study, the sample size calculation was performed on the basis of cervical dilatation of <2 cm being the definition of early detection of preterm labor and assuming that 60% of monitored patients would have early detection compared with only 30% of not monitored patients. Alpha was 0.05 and β was 0.20 (power = 80%). Thus 48 patients with preterm labor per group would be required. It was further assumed that 30% of the enrolled highrisk patients would have preterm labor so that a total enrollment of 320 would be required.

Statistical analysis. Two group comparisons were made with t tests or Wilcoxon rank sum tests, depending on frequency distributions for continuous variables. χ^2 tests or Fisher's exact probabilities (depending on number of categories and cell counts) were done for categorical variables. Three group comparisons were made

Table I. Characteristics of subjects at entry to the study

	All enrollees (n = 377)			Subgroup with preterm labor $(n = 82)$		
	Monitored (n = 198)	Not monitored $(n = 179)$	Monitored (n = 43)	Not monitored $(n = 39)$		
Demographic						
Maternal age (yr, mean ± SD)	27.0 ± 5.1	26.3 ± 5.2	26.7 ± 4.9	26.7 ± 5.6		
White (%)	53.5	47.6	45.2	44.7		
High school graduate (%)	73.9	68.9	73.8	69.2		
Substance use						
Alcohol (%)	5.1	7.3	4.7	7.7		
Cocaine (%)	5.6	8.9	7.0	5.1		
Heroin (%)	0.5	1.1	2.3	2.6		
Cigarette use (%)	21.2	27.9	25.6	33.3		
Obstetric						
Prior pregnancies (No.)	2.6 ± 1.7	2.8 ± 1.8	2.6 ± 1.5	3.0 ± 1.9		
Live births (No.)	1.7 ± 1.2	1.8 ± 1.3	1.7 ± 1.2	2.0 ± 1.3		
Spontaneous abortions (No.)	0.5 ± 0.9	0.6 ± 1.0	0.5 ± 0.8	0.6 ± 0.9		
Elective abortions (No.)	0.3 ± 0.7	0.3 ± 0.6	0.3 ± 0.6	0.2 ± 0.4		
Weeks' gestation at entry	26.6 ± 2.8	26.4 ± 2.9	26.6 ± 2.8	25.8 ± 2.7		
Previous preterm birth (%)	45.0	54.8	53.5	71.8		
Twin gestation (%)	12.1	7.8	20.9	20.5		
2nd Trimester abortion (%)	11.1	12.8	11.6	10.3		
Threatened labor (%)	16.6	11.1	11.6	10.3		
Creasy score, median	15	15	15	17		
Creasy score, extremes	10-41	10-32	10-37	10-34		

with analysis of variance or χ^2 depending on the type of data. Two-tailed probability or ≤0.05 was considered significant. No adjustments were made for multiple comparisons.

Results

Comparison of groups at entry. The demographic and medical characteristics of the monitored and not monitored groups are summarized in Table I. At entry to the study no significant differences were observed between the two groups for any of these characteristics. This was true for both the 377 patients originally randomized and the subgroup of 82 who went on to have preterm labor. In addition, since randomization was stratified by site and twins, it was verified that within each randomization no significant differences were observed between the monitored and not monitored groups in any of their medical and demographic characteristics.

Comparison of groups after entry. Several analyses were performed to verify that the two groups received similar care after entry to the study. There were no significant differences between the two groups. The number of routine visits per patient among the monitored and the not monitored patients was 7.1 and 6.7, respectively. For nonroutine visits the monitored patients averaged 2.0 visits and not monitored patients averaged 1.8 visits. Fig. 2 illustrates the number of routine and nonroutine visits for the two groups during each week of gestation that the patients participated in the study.

The incidence and timing of preterm labor was also similar for the two groups. Preterm labor was detected in 43 of 174 (24.7%) monitored patients and in 39 of 160 (24.4%) not monitored patients (Fig. 1). The average gestational age at diagnosis of preterm labor was 32.9 weeks for both groups (Table II).

Cervical dilatation at diagnosis of preterm labor. The primary end point for the study was the timing of detection of preterm labor as measured by the cervical dilatation at the time of diagnosis. Cervical dilatation data were available for 77 of the 82 subjects who had preterm labor. Dilatation data were not recorded at the time of diagnosis of preterm labor for two monitored and two not monitored subjects. Dilatation data for one not monitored subject were not used because a cerclage was in place at the time of the diagnosis of preterm labor.

Women in the monitored group had significantly less cervical dilatation at the time of diagnosis of preterm labor (Table II). The mean dilatation for the monitored group was 1.4 cm compared with 2.5 cm in the not monitored patients (p = 0.0006). Some patients had cervical dilatation before the onset of preterm labor; therefore the change in dilatation from the cervical examination immediately prior to the onset of preterm labor also was studied. Women in the monitored group had a mean change in cervical dilatation of 1.1 cm

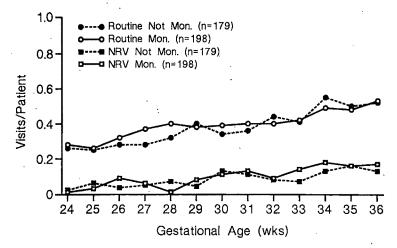


Fig. 2. Number of routine and nonroutine visits per patient per week of gestation for entire study population. *Routine*, Scheduled prenatal visits; *NRV*, nonroutine prenatal visits; *not mon.*, not monitored patients; *Mon.*, monitored patients.

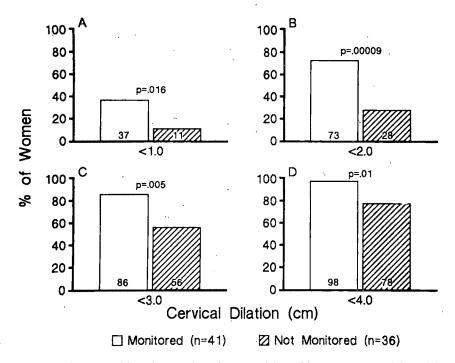


Fig. 3. Percent of women with early detection of preterm labor with <1.0 cm (A), <2.0 cm (B), <3.0 cm (C), and <4.0 cm (D) used as cutoffs for early detection of preterm labor.

compared with 2.0 cm in not monitored women (p = 0.004).

In addition to evaluating the overall distribution of cervical dilatation, the likelihood of early detection of preterm labor was also determined. The initial sample size calculation for the end point of cervical dilatation was based on the definition of early detection of preterm labor being a cervical dilatation of <2 cm, and it was assumed that 60% of monitored patients would have early detection compared with only 30% of not monitored patients. Detection of preterm labor before

2 cm cervical dilatation was actually accomplished in 73:1% of monitored subjects compared with only 27.8% of not monitored subjects (p=0.00009) (Fig. 3, B). Since clinicians may vary with regard to their definition of early detection of preterm labor, Fig. 3 shows the group comparisons with four different cutoff values (1, 2, 3, and 4 cm) for early detection. The monitored group had significantly earlier detection than the not monitored group for all four cutoff values.

Since patients with preterm labor represent a subset of the original randomized groups, multiple regression

Table II. Obstetric outcomes for women with preterm labor

	$Monitored \\ (n = 43)$	Not monitored $(n = 39)$	p Value
Gestational age at diagnosis of preterm labor (wk)		_	<u> </u>
Mean \pm SD	32.9 ± 3.2	32.9 ± 2.9	
Cervical dilatation at diagnosis of preterm labor (cm)*			
Mean ± SD	1.4 ± 1.3	2.5 ± 1.5	0.0006
Median (extremes)	1.0 (0-6.5)	2.0 (0-6.0)	0.0002
Change in cervical diagnosis from prior examination (cm)*		(5 227)	
Mean ± SD	1.1 ± 1.2	2.0 ± 1.4	0.004
Median (extremes)	1.0 (0-6.5)	2.0 (0-5.0)	0.001
Duration of gestation after diagnosis of preterm labor (wk)†	` '	(, , ,	
Mean ± SD	3.7 ± 3.8	2.0 ± 2.9	0.02
Gestational age at delivery (wk)†			
Mean ± SD	36.6 ± 2.4	34.9 ± 3.2	0.009

On the rows where data are summarized with mean, data analysis was with Student t tests. On the rows where data are summarized with median, data analysis was with Wilcoxon rank sum test.

Table III. Neonatal outcomes for singleton gestations with preterm labor*

•	Monitored $(n = 33)$	Not monitored $(n = 30)$	p Value
Birth weight (gm)†		-	
Mean ± SD	2934 ± 708	2329 ± 733	0.002
<2500 gm (%)	19	63	0.0007
<2000 gm (%)	10	37	0.02
Neonatal intensive care			
No. of infants	5	13	0.02
Total days	50	324	
Oxygen therapy		•	
No. of infants	0	7	0.004
Total days	0 .	68	
Mechanical ventilation			
No. of infants	. 0	5	0.02
Total days	0	54	

^{*}Medically indicated preterm deliveries (one in each group) were excluded from these analyses.

analysis was performed with dilatation at diagnosis of preterm labor as the dependent variable and numerous independent variables, such as obstetric history variables including substance use, study site, and monitoring versus not monitoring. The only variable that had a significant independent effect on dilatation at diagnosis was use of the monitor, which was associated with a 0.9 cm decrease in cervical dilatation (p = 0.01). Cohort analyses were done for women who entered the study at <25 weeks' gestation, at ≥27 weeks, and at ≥29 weeks' gestation. Mean cervical dilatation and cervical change at the diagnosis of preterm labor was less in the monitored group for all of these cohorts.

Pregnancy and neonatal outcome. To evaluate the clinical importance of the early detection of preterm labor observed in the monitored group, duration of gestation after diagnosis of preterm labor (Table II), infant birth weight, and measures of neonatal morbidity (Table III) were compared between the two groups.

The mean duration of gestation after diagnosis of preterm labor was 3.7 weeks in 41 monitored patients (one patient did not have gestational age at delivery recorded and one was excluded because of medically indicated preterm delivery) compared with only 2.0 weeks in 38 not monitored patients (one excluded because of medically indicated preterm delivery [p = 0.02]) (Table III). Therefore the mean gestational age at delivery was 36.6 weeks in the monitored group compared with only 34.9 in the not monitored group (p = 0.009) (Table II). These gestational duration data do not significantly change if the two medically indicated preterm deliveries are included in the analysis. Multiple regression analysis also was performed with gestational age at delivery as the dependent variable and numerous independent variables including age, race, Creasy score, substance use variables, maternal

^{*}Dilatation data were not available for two monitored and three not monitored subjects.

[†]Gestational age at delivery was not available for two monitored and one not monitored subjects.

[†]Birth weight data were not available for two monitored infants who were born at term.

history variables, study site, monitoring versus not monitoring, and cervical dilatation at diagnosis of preterm labor. The only variable that had a significant independent effect on gestational age at delivery was cervical dilatation at diagnosis of preterm labor, which was associated with a 0.6-week decrease in gestational age at delivery for each centimeter increase in dilatation (p = 0.03).

Birth weight and neonatal morbidity variables were evaluated after exclusion of 17 (nine monitored and eight not monitored) twin gestations and two (one in each group) medically indicated preterm deliveries. In addition, there were two patients for whom birth weight data were not available. Twin gestations were excluded from these analyses because of the potential effects of twinning on birth weight and neonatal morbidity.

Birth weight of singletons differed significantly between the monitored and not monitored groups. The mean birth weight of infants from the monitored group (n = 31) was 2934 gm compared with only 2329 gm for the not monitored group (n = 30) (p = 0.002). Only 19% of infants from the monitored group weighed <2500 gm versus 63% of the not monitored babies (p = 0.0007). The incidence of infants weighing <2000 gm was only 10% for the monitored group compared with 37% for the not monitored group (p = 0.02) (Table III). Singleton infants born to monitored women also suffered significantly less neonatal morbidity as measured by need for neonatal intensive care, oxygen therapy, and mechanical ventilation (Table III). Only five infants (50 total neonatal intensive care unit days) of the 33 monitored singleton gestations required admission to the neonatal intensive care unit (p = 0.02). None of the 33 infants who were products of monitored singleton gestations required oxygen therapy or mechanical ventilation compared with seven infants who required oxygen therapy, for a total of 68 days (p = 0.004), and five infants who required mechanical ventilation for a total of 54 days (p = 0.02) among the 30 products of not monitored singleton gestations (Table III).

There were 252 patients who were randomized into monitored and not monitored groups who did not experience preterm labor outcome. Data were available on 250 patients. The mean gestational age at delivery of the controls without preterm labor was 39.1 weeks (± 2.3 weeks SD). The mean gestational age at delivery of the monitored group without preterm labor was 38.8 weeks (± 2.1 weeks SD), p=0.28. Likewise, the birth weights of the singletons in the control group without preterm labor was 3154 gm (\pm 654 gm SD) and the birth weights of singletons in the monitored group was 3269 gm (\pm 604 gm SD), p=0.18.

Comment

This study demonstrates that objective assessment of uterine activity, by home uterine activity monitoring in women at high risk for preterm labor, allows detection of preterm labor at less advanced cervical dilatation. The clinical importance of this earlier detection of preterm labor is supported by the increased gestational duration after diagnosis of preterm labor and the resultant improved gestational age at delivery, increased birth weight, and decreased neonatal morbidity observed in the monitored group.

This report represents the largest published, prospective, multicenter, randomized, and blinded evaluation of the efficacy of home uterine activity monitoring for earlier detection of preterm labor in high-risk pregnancies. Previously published studies have suggested beneficial effects of home uterine activity monitoring^{3, 5, 6}; however, the study designs used did not allow for the separation of the impact of the use of the monitor from the impact of the additional perinatal nursing support.7, 10 Some studies3, 6 did not provide comparison groups receiving the same perinatal center obstetric care. The studies that failed to show beneficial effects of home uterine activity monitoring have not focused on the value of the device when it is used without additional nursing care.11.12 Also, the present study differs from others in using twice-daily monitoring and transmission of data, rather than once-daily transmission of monitor strips:

When one is evaluating a new device, it is essential to separate clearly those effects that are a result of the information derived from the use of the device from those effects of other nonstandard interventions. Previous studies have evaluated daily perinatal nursing support used along with home uterine activity monitoring. The current report evaluates the impact of the addition of objective assessment of uterine activity to high-risk obstetric care on pregnancy outcome. In this study design, (1) no routine daily perinatal nursing support was provided to either group, (2) all study patients were provided with identical high-risk obstetric care and education in self-palpation of uterine contractions (as described by Herron et al.18), (3) caretakers were blinded to subject's group assignment, (4) preterm labor was strictly defined, (5) all subjects were included in the data analysis on the basis of their original group assignment regardless of compliance, and (6) the primary end point was cervical dilatation at diagnosis of preterm labor, since other variables (e.g., incidence of preterm birth) are strongly related to the incidence of preterm labor in the study population and to the effectiveness of tocolytic therapy.

Although caretakers were blinded to the subjects' group assignments, it is not possible to be absolutely certain that all examiners were indeed unaware of a patient's group assignment. It is estimated that not more than six women were examined by caretakers who were aware of the patient's group assignment. The results of the study did not change when these patients were excluded from the data analysis.

The study did not include a sham monitoring group, and therefore it is not possible to determine the independent effects of the four procedures that are an integral part of the monitoring protocol. These procedures, which represent the only known differences between the two groups, include (1) wearing a tocodynamometry belt during each recording session, (2) relative inactivity during each recording session, (3) data transmission after each recording session, and (4) patient notification if four or more contractions were observed. Although it is possible that any of these four procedures may have contributed to the results, detection and notification of increased contractions is the most reasonable explanation for the early detection of preterm labor observed in the monitored group. There were similar outcomes in the control and monitored group among patients who did not go into preterm labor, which also makes the twice daily monitoring unlikely to affect the course of the pregnancies except for diagnosing preterm labor at an earlier cervical dilatation.

The benefit of monitoring appears to be in the earlier detection of preterm labor, before perception by the patient and before significant cervical dilatation has occurred. Self-palpation of uterine contractions had been shown to be less sensitive than tocodynamometry in detecting uterine activity.2, 14 Objective quantitation of contractions is a useful method of surveillance for preterm labor and detects contractions before women perceive them.2 Since progression of labor occurs over hours rather than days or weeks one would not expect the gestational age at diagnosis of preterm labor to differ significantly between the groups as shown in this study, with mean gestational age at diagnosis of preterm labor at 32.9 weeks in the monitored and not monitored groups. The observation that there were no differences between the groups for the gestational age at the time of diagnosis of preterm labor and the incidence of preterm labor makes it unlikely that overdiagnosis of preterm labor or lead time bias could account for the results of the study. This protocol did not address specific tocolytic regimens. Once preterm labor was diagnosed, each center continued its usual protocols. Therefore it is not possible to assess the relative effects of various medical interventions on the outcomes. The fact that the vast majority of caretakers were unaware of the patient's group assignment makes it unlikely that there were significant differences between the medical interventions received by the two groups. In addition, the two groups were medically and demographically similar at entry into the study and at the time of diagnosis of preterm labor. They had similar numbers of routine and nonroutine obstetric visits, incidence of preterm labor, similar gestational age at diagnosis of preterm labor, and were similar in the relationship between dilatation at diagnosis of preterm labor and subsequent duration of gestation.

Zlatnik¹⁵ and King et al. ¹⁶ have shown that long-term tocolysis with significant prolongation of gestation is possible if preterm labor is diagnosed before advanced cervical dilatation. Multiple regression analysis confirmed that earlier detection of preterm labor in the monitored group led to significant prolongation of gestation. Although there is disagreement as to the specific definition of advanced cervical dilatation, a significantly higher percentage of the monitored patients in this study had early detection of preterm labor, as measured by cervical dilatation, whether <1, 2, 3, or 4 cm dilatation was used as the cutoff for early detection. The result was improved gestational age at delivery and increased birth weight of infants in the monitored group.

A medically and sociodemographically diverse population from three perinatal sites participated in this study. Subgroup analyses were performed with specific medical risk factors, gestational ages at entry, gestational ages at diagnosis of preterm labor, and compliance with study protocol. The results of these analyses showed that the information derived from home uterine activity monitoring appeared to be similarly beneficial for all patients who used the monitor. The subset of the monitored group (n = 12) who did not comply with the protocol had results that were similar to those of the not monitored group. Prior preterm delivery was the only medical risk factor for preterm labor for which there were a sufficient number of patients for separate analysis. These data were consistent with the data for the entire study population showing beneficial effects for all outcome variables. On the basis of these study data, the United States Food and Drug Administration has given approval for use of this device in women with a history of preterm birth.17 Although none of the other individual medical risk factors had a sufficient sample size for separate analyses, when considered as one group (containing the 31 patients without a history of preterm birth), the monitored group showed significant beneficial effects similar in magnitude to the subset of women with a history of preterm birth. Women with other medical risk factors for preterm labor may therefore also benefit from home uterine activity monitoring. Future studies may further address the benefit in women with other specific maternal risk factors.

Our study demonstrates that home uterine activity monitoring, when used in selected high-risk women as the sole addition to standard high-risk care, results in earlier detection of preterm labor. This led to an impressive mean 1.7-week improvement in gestation compared with not monitored women, as well as a mean 605 gm greater birth weight.

In summary, this study demonstrated that use of home uterine activity monitoring, in selected high-risk patients, significantly increases the likelihood of diagnosis of preterm labor before advanced cervical dilatation, thus providing the clinician with a better opportunity to initiate tocolytic therapy directed at improving pregnancy outcome.

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Adjunctive clindamycin therapy for preterm labor: Results of a double-blind, placebo-controlled trial

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A double-blind, placebo-controlled, randomized trial was conducted to evaluate the efficacy, safety, and tolerance of a course of clindamycin (administered for 3 days intravenously and 4 days orally) among hospitalized women with preterm labor at ≤34 weeks' gestation who were treated with tocolytics. One hundred three woman-perinate pairs were analyzed. Univariate analysis demonstrated that pregnancies were continued longer in women treated with clindamycin than in women who received placebo (clindamycin-treated group, 35 days; placebo-treated group, 25 days; p = 0.02). Survival analysis showed that pregnancy continued at least 35.5 days in 50% of clindamycin-treated women versus 20 days for control women (p = 0.03). Obstetric and microbiologic parameters associated with treatment outcomes were also sought. Women with bacterial vaginosis more often delivered preterm (p = 0.03; relative risk, 1.4; 95% confidence interval, 1.04 to 2.0). Among women with bacterial vaginosis, trends for increased duration of pregnancy (clindamycin-treated group, 36 days; placebo-treated group, 19 days), increased birth weight (clindamycin-treated group, 2634 gm; placebo-treated group, 2256 gm), and increased mean gestational age at delivery (clindamycin-treated group, 35 weeks; placebo-treated group, 34 weeks) were associated with clindamycin treatment. Women with either group B streptococcus, Chlamydia trachomatis, Trichomonas vaginalis, or Staphylococcus aureus were more likely to have preterm premature rupture of membranes (p = 0.01). Clindamycin treatment of these women reduced the incidence of preterm premature rupture of membranes to that of uninfected subjects. Stratification by gestational age at enrollment showed clindamycin treatment to be associated with an increased interval to delivery only among mothers enrolled before 33 weeks' gestation (clindamycin-treated group, 40 days; placebo-treated group, 28 days; p < 0.05). Treatment with clindamycin appeared safe and well tolerated, with benefits limited to women who were ≤32 weeks' gestation. (Am J OBSTET GYNECOL 1991;165:867-75.)

Key words: Preterm labor, antibiotics, infection, bacterial vaginosis

Preterm birth remains a leading cause of infant morbidity and mortality. In spite of much effort to identify and provide intensive antenatal surveillance to women at risk, the occurrence of preterm birth in the United States appears to have increased between 1985 and 1987.1 Considerable information suggests that maternal reproductive tract infection and associated inflammation may play initiating or contributory roles in the pathogenesis of preterm labor and birth for significant numbers of pregnant women.2 Effective antibiotic treatment of undetected maternal reproductive tract infection may allow for prolongation of pregnancy and may improve outcome for women with preterm labor. Previous trials of antimicrobial therapy in addition to tocolytic therapy for women in preterm labor have yielded encouraging but inconsistent results.3-6 Differ-

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ences in study design, populations, and/or prevalence of reproductive tract infections and antibiotic selection may contribute to such differences.

In this study we evaluated use of clindamycin as adjunctive antimicrobial treatment for women hospitalized with idiopathic preterm labor to evaluate possible pathogenic roles for reproductive tract infection in preterm birth and to identify women most likely to benefit from such antimicrobial treatment.

Material and methods

Between July 1986 and April 1989, women who were hospitalized at ≤34 weeks' gestation and who received parenteral tocolytic agents for idiopathic preterm labor were asked to participate in this double-blind, placebocontrolled trial. Each participant completed an informed consent form approved by the human subjects committee of the University of Colorado Health Sciences Center or the Denver Department of Health and Hospitals.

Patients were excluded from the study for the following reasons: (1) the presence of a recognized cause of preterm labor or obstetric complication, such as placenta previa, multiple gestation, abruptio placentae, cervical cerclage, known uterine or fetal anomaly, pregnancy-induced hypertension, or premature rupture of membranes; (2) known or suspected infection necessitating treatment, e.g., clinically recognized chorioamnionitis, urinary tract infection, or pneumonia; (3) fetal indication for delivery; (4) clinically significant maternal cardiac, respiratory, liver, renal, or immunologic disease; (5) use of antibiotics within 2 weeks before the start of the study; (6) a history of diarrhea or colitis; (7) sensitivity or intolerance to clindamycin.

On enrollment, medical, obstetric, and behavioral information was recorded. A clean-voided urine sample was collected for urinalysis and culture. A blood sample for complete blood count with differential count was obtained. An examination was performed with a sterile vaginal speculum; separate endocervical samples were immediately inoculated onto a chocolate agar and a modified Thayer-Martin split plane for recovery of Neisseria gonorrhoeae and into sucrose-phosphate glutamate transport media for recovery of Chlamydia trachomatis. Samples from the cervix and upper vagina were inoculated onto 5% sheep blood agar (tryptic soy base, Remel, Denver) for recovery of group A streptococcus, group B streptococcus, Staphylococcus aureus, Gardnerella vaginalis, and yeast species. Additional lateral vaginal wall samples were inoculated in standard mycoplasma medium for recovery of Mycoplasma hominis and Ureaplasma urealyticum and were placed in 1 ml of saline solution for microscopic examination for motile trichomonads and yeast pseudohyphae. Release of amine odor ("whiff test") from a sample of vaginal fluid was tested by the addition of 10% potassium hydroxide. Another sample of vaginal fluid was allowed to air dry on a slide for Gram's stain evaluation for bacterial vaginosis and Mobiluncus sp. bacterial morphotypes.7 Microbiologic cultures were processed and microorganisms were identified by means of standard techniques.8,9

After laboratory specimens were obtained, women in the study were randomly assigned according to a computer-generated random number list to receive either intravenous clindamycin 900 mg every 8 hours for nine doses or a similarly packaged and labeled saline solution placebo. Intravenous therapy was followed by treatment with oral clindamycin 300 mg four times daily for 4 days or with an identical-appearing lactose placebo to complete 1 week of therapy. Intravenous study treatment was prepared and double blinded by the hospital pharmacy. Corresponding oral study treatment was prepared and double blinded by The Upjohn Company, Kalamazoo. Women discharged from the hospital before completion of oral treatment were given the study bottle and a medication diary in which to record medications taken at home. Undelivered subjects were seen in the antenatal clinic by one of the investigators 2 to 4 weeks after the completion of study treatment.

At this time medication bottles and diaries were collected. Women were questioned regarding side effects, and all initial microbiologic sampling was repeated. Participants continued their care in a specialized obstetric clinic. Follow-up information was obtained by review of maternal and newborn records through 6 weeks post partum and 3 months of life, respectively.

All participants received parenteral tocolytic therapy (subcutaneous or intravenous terbutaline, intravenous magnesium sulfate, or intravenous ritodrine, or a combination of these medications). Tocolytic therapy and study treatment were discontinued if labor persisted and delivery was considered clinically inevitable or if evidence of maternal intolerance, fetal distress, or preterm rupture of membranes occurred. Successful tocolysis was followed by oral tocolytic therapy with either terbutaline or nifedipine until 37 weeks' gestation was achieved or until findings of recurrent labor necessitating parenteral treatment were noted. Subsequent episodes of preterm labor were treated according to obstetric protocols without further study therapy. Amniocentesis was not specifically performed as part of this protocol. Betamethasone (12.5 mg each 24 hours for two doses, intramuscularly) was administered to the mother if clinically indicated. Obstetric care providers were informed if subjects were colonized with group B streptococcus; neonatal care providers were informed when C. trachomatis or group B streptococcus was recovered. Women in whom group B streptococcus was identified were given intravenous ampicillin when active labor began or when membranes ruptured.

Gestational age at enrollment was determined by a combination of "best" overall obstetric criteria,10 including last menstrual period, detection of fetal heart rate with fetoscope, fundal height measurements, and ultrasonographic measurements. After delivery, overall gestational age was determined by combining those criteria with newborn examination.11 Preterm labor was defined as two or more uterine contractions in 20 minutes that persisted after bed rest and intravenous hydration and required administration of parenteral tocolytic therapy because of change in cervical dilatation or effacement. Birth at <37 completed weeks' gestation was considered preterm. Premature rupture of membranes was defined as rupture of membranes that occurred after successful tocolysis and >1 hour before the onset of uterine contractions leading to birth. Chorioamnionitis was based on clinical criteria that included maternal intrapartum fever ≥38° C and two or more of the following: maternal tachycardia, fetal tachycardia, maternal leukocytosis, or uterine tenderness. Diagnosis of postpartum endometritis was determined by findings of fever, uterine tenderness, and foul or purulent lochia.

Before study initiation, it was determined that a sam-

ple containing 57 patients in each group would detect a 25% increase in the number of women achieving 37 weeks' gestation with a power of 80% and at the 0.05 significance level. This was based on the assumption that 50% of women receiving tocolytic therapy are delivered at term. 12 Clindamycin was selected as the study agent principally because of its relatively focused antimicrobial activity against several microorganisms implicated in preterm birth (group B streptococcus, genital mycoplasmas, C. trachomatis, and aerobic and anaerobic microflora associated with bacterial vaginosis) and because of the relative ease and safety of both intravenous and oral administration. In a previous trial3 we demonstrated that oral administration of erythromycin was associated with prolongation of pregnancy only among women with cervical dilatation ≥1 but ≤4 cm; several women were intolerant of oral erythromycin treatment. A review of antibiotic-associated diarrhea and pseudomembranous enterocolitis at the study sites showed that clindamycin use was not associated with increased occurrence of diarrhea in comparison with other antibiotics. Clindamycin use among newborns of varying gestational ages is well described.13

Data were summarized with descriptive statistics. Treatment group differences for categoric variables were evaluated by means of χ^2 tests, and normally distributed, continuous data were evaluated with twotailed Student's t test. Nonparametric or skewed data were evaluated with Wilcoxon's rank sum test. Survival analysis (SAS LIFETEST Procedure; SAS, Cary, N.C.) was performed to compare treatment group differences for time gained from study entry to delivery. The effect of treatment on microbiologic findings was evaluated with McNemar's χ^2 test for paired data. To control for potentially confounding effects of treatment and microorganisms, data were stratified according to presence of bacterial vaginosis and other susceptible microorganisms. Multiple regression analysis was performed to control effects of possible confounding variables in the prediction of days from study entry to delivery. Finally, logistic regression was performed with BMDP Statistical Software (Los Angeles) to control for the effects of potentially confounding variables on the outcome variable preterm birth.

Results

One hundred seventeen women were enrolled in this study. Fourteen subjects were excluded from analysis before the unblinding of the treatment code and data analysis. Fetal distress developed in one patient who underwent an emergent cesarean delivery before she received study treatment. Two patients withdrew consent, one before study treatment and one after an apparent allergic reaction to the initial dose. Three patients were withdrawn from the study when clinical

chorioamnionitis was detected; each had received a single dose of study treatment. One patient was delivered of previously undetected twins. Six subjects who were enrolled in spite of having exclusion criteria were eliminated from analysis. A final patient was excluded as a result of pharmacy error. All other patients were evaluated regardless of the number of doses they received. The total sample evaluated included 103 women and their newborns; 53 received clindamycin and 50 patients received placebo.

During study drug treatment, a single patient received ampicillin for group B streptococcus prophylaxis as a result of refractory labor, and another received ampicillin because of preterm rupture of membranes; each patient had received placebo. None of the four clindamycin-treated women in whom group B streptococcus was identified received additional antibiotics during study treatment. Ampicillin was given to those women when active labor began (10 to 59 days) after enrollment or if they had premature rupture of membranes. One patient in each study group received oral amoxicillin because of bronchitis or otitis media during study treatment; another patient who received placebo also was treated with oral metronidazole for G. vaginalis bacteriuria. The two remaining women with bacteriuria at study enrollment received nitrofurantoin. Each of these patients who received a nonstudy antibiotic was included in the analysis.

There were no differences among the multiple demographic, obstetric, or behavioral parameters for women who received clindamycin or placebo (all data not shown; Table I). Mean gestational age at enrollment did not differ between treatment groups. Thirty-nine clindamycin patients (73.6%) and 32 placebo patients (64.0%) were ≤32 weeks' gestation at enrollment. There were no differences for mean Bishop's scores at enrollment, duration of parenteral tocolysis, or types of tocolytic used for clindamycin- and placebo-treated women. Ninety-eight percent of both treatment groups received terbutaline; 66% of clindamycin-treated subjects and 58% of women given placebo received magnesium sulfate. Fewer women in each treatment group were given ritodrine (four clindamycin, three placebo) or nifedipine (three clindamycin, six placebo) for tocolysis. All subjects received parenteral tocolytic therapy for at least 24 hours unless delivery occurred within the first study day. The majority of subjects were multiparous. Among multigravid clindamycin-treated women, 62.2% had experienced a prior preterm birth, compared with 44% of multigravid women randomized to placebo (p = 0.2).

Thirty-eight percent of clindamycin-treated women and 42% of placebo-treated women experienced lesser medical problems during their antenatal care before study enrollment. Medical conditions were well con-

Table I. Selected maternal demographic, behavioral, and obstetric characteristics at study enrollment

	Clindamycin .	
	(n=53)	$Placebo\ (n=50)$
Demographic	·	
Age*	$22.2 \pm 4.2 (15-33)$	$21.5 \pm 4.6 (16-37)$
<18 yr	5/53 (9%)	8/50 (16%)
18-29 yr	43/53 (81%)	39/50 (78%)
≥30 yr	5/53 (9%)	3/50 (6%)
Ethnic group or ancestry		
Northern European	34/53 (64%)	35/50 (70%)
African American	9/53 (17%)	6/50 (12%)
Hispanic surname	10/53 (19%)	8/50 (16%)
Asian	0	1/50 (2%)
Education		
<12 yr	26/52 (50%)	20/49 (41%)
≥12 yr	26/52 (50%)	29/49 (59%)
Behavioral		
Smoked		
None	30/53 (57%)	26/50 (52%)
≥½ pack/day	12/53 (23%)	10/50 (20%)
Alcohol use (any/wk)	6/53 (11%)	7/50 (14%)
Street drugs (any)†	9/52 (17%)	4/50 (8%)
Obstetric history		
Primiparous patients	16/53 (30%)	25/50 (50%)‡
Parity	$1.2 \pm 1.1 (0-4)$	$0.9 \pm 1.1 (0-4)$
Antenatal history		•
Prior vaginal bleeding	15/53 (28%)	16/50 (32%)
No prior prenatal care	8/49 (16%)	3/48 (6%)
Gestational age at enrollment (wk)*	$30.5 \pm 2.8 (23-34)$	$31.0 \pm 2.6 (24-34)$
Bishop's score*	$5.8 \pm 2.6 (0-10)$	$6.4 \pm 2.4 (2-11)$

^{*}Mean ± SD. Range is shown in parentheses.

trolled and included asthma (two patients), gestational diabetes (seven patients), hypothyroidism (one patient), pyelonephritis (one patient), anemia (22 patients), and seizure disorder (two patients). In addition, three women were monitored for intrauterine growth retardation and two for isoimmunization. Two women had experienced hyperemesis gravidarum.

Maternal microbiologic findings are shown in Table II. There were no study group differences for any of the microorganisms recovered at enrollment. Two to 4 weeks after the completion of study treatment, 31 clindamycin subjects (58.5%) and 26 placebo patients (52.0%) were reexamined for microbiologic findings. Clindamycin treatment was associated with significant reduction in recovery of *M. hominis* and with the identification of bacterial vaginosis. The number of women in whom *G. trachomatis, Mobiluncus* sp. bacterial morphotypes, *S. aureus*, and group B streptococcus were identified was too small to allow for evaluation of treatment effects. Recovery of yeast species was not increased after clindamycin treatment (Table II).

Amniotic fluid was obtained from 31 clindamycin patients (58.5%) and 24 placebo patients (48.0%). Thirty-nine percent of clindamycin patients and 46% of placebo patients had polymorphonuclear leukocytes noted on Gram's stain evaluation of amniotic fluid samples. One amniotic fluid culture was positive in each

group (clindamycin-treated group, nonhemolytic streptococcus; placebo-treated group, Fusobacterium necrophorum).

Overall, preterm birth (<37 weeks' gestation) occurred among 62% of the enrolled women. Univariate analysis showed that pregnancies continued significantly longer in clindamycin-treated women than in those receiving placebo (clindamycin-treated group, mean 35.3 days and range 0 to 115; placebo-treated group, mean 25.4 days and range 1 to 77; p=0.02; Table III). Survival analysis demonstrated that pregnancies continued at least 35.5 days in 50% of women treated with clindamycin but 50% of placebo patients had been delivered by 20 days (p=0.03) (Fig. 1).

When gestational age at enrollment was controlled by stratification (\leq 32 and 33 to 34 weeks' gestation), univariate analysis showed that clindamycin treatment was associated with a prolonged mean interval from study entry to delivery only among women enrolled before 33 weeks' gestation (enrolled \leq 32 weeks; clindamycin-treated group, 40.4 days; placebo-treated group, 29.0 days; p < 0.05; enrolled 33 to 34 weeks: clindamycin-treated group, 21.0 days; placebo-treated group, 18.9 days, p = 0.7).

Stepwise multiple regression analysis with backward elimination was performed; independent variables included study treatment, bacterial vaginosis, gestational

[†]A single clindamycin patient reported use of "crack" cocaine.

p = 0.06.

Table II. Enrollment and reexamination microbiologic findings for clindamycin and placebo patients*

		Clina	lamycin			Pla	cebo	
	Enrollment $(n = 53\dagger)$		Reexamination $(n = 31)$		Enroll (n =		Reexami (n =	
	No.	%	No.	%	No.	%	No.	%
Bacterial vaginosis	15/52	28.8	2/29	6.9‡	10/50	20.0	6/26	23.1
Mobiluncus sp. morphotypes	2/52	3.8	()	3/47	6.4	3/26	11.5
M. hominis	18/51	35.3	1/30	3.3§	12/48	25.0	7/24	29.2
G. vaginalis	21/49	42.9	6/30	20.0	16/47	34.0	12/24	50.0
U. urealyticum	37/51	72.5	26/30	86.7	38/48	79.2	19/24	79.2
C. trachomatis	2/52	3.8	1/28	3.6	2/48	4.2	2/25	8.0
T. vaginalis	1/53	1.9	() .	1/50	2.0	0	
S. aureus	2/49	4.1	()	2/47	4.3	0	
Group B streptococcus	4/53	7.5	1/31	3.2	3/48	6.2	0	
Yeast sp.	11/50	22.0	9/30	30.0	9/47	19.1	6/24	25.0
Bacteriuria	1/50	2.0	ND		2/47	4.3	ND	

ND, Not determined.

Table III. Pregnancy and neonatal outcomes for clindamycin and placebo patients

	Clindamycin	Placebo	p Value
Days from study entry to delivery	35.3 ± 24.1 (0-115)*	25.4 ± 20.0 (1-77)	0.02
Gestational age at birth (wk)	$35.4 \pm 3.3 (25-41)$	$34.9 \pm 3.3 \ (25-42)$	NS
Birth weight	$2586.2 \pm 643.0 (815-3780)$	$2441.0 \pm 694.2 (780-4280)$	NS
Preterm birth (<37. wk)	33/53 (62.3%)	31/50 (62.0%)	NS
Preterm birth (≤32 wk)	8/39 (20.5%)	7/32 (21.9%)	NS
Preterm premature rupture of membranes	5/53 (9.4%)	7/50 (14.0%)	NS
Low birth weight (<2500 gm)	21/53 (39.6%)	26/50 (52%)	NS
Readmissions for preterm labor	19/44 (43.2%)	14/39 (35.9%)	NS
Clinical chorioamnionitis	1/53 (1.9%)	3/50 (6.0%)	NS
Postpartum endometritis	4/36 (11.1%)	4/35 (11.4%)	NS
Newborn treatment to rule out sepsis	16/53 (30.2%)	14/50 (28.0%)	NS
Pneumonia	1/52 (1.9%)	3/50 (6.0%)	NS
Days in level II or III nursery	$7.1 \pm 11.6 (8-38)$	$16.8 \pm 51 (0-336)$	0.2
Neonatal deaths	2/53 (3.8%)	0	NS
Sudden infant death syndrome	1/51 (1.9%)	1/50 (2.0%)	NS

NS, Not significant.

age at enrollment, cervical dilatation, and cervical effacement (Table IV). Increased cervical dilatation (p < 0.0001) and effacement (p = 0.09) at enrollment and gestational age at enrollment (p < 0.008) were associated with reduced time from enrollment to delivery. It is important to note that prolongation of pregnancy associated with clindamycin treatment persisted after controlling for the effects of cervical dilatation, effacement, and gestational age at enrollment. The presence of bacterial vaginosis, C. trachomatis, group B streptococcus, T. vaginalis, or S. aureus was not significantly related to number of days gained from study entry to delivery when multivariate analysis was used.

Newborns of mothers treated with clindamycin re-

quired a mean of 7.1 days of level II or III nursing care versus 16.8 days for those whose mothers received placebo (NS) (Table III). There were no statistically significant differences for birth weight (mean weight: clindamycin-treated group, 2586 gm; placebo-treated group, 2441 gm), gestational age at birth, number of women delivered before 37 or ≤32 weeks' gestation, the number of low-birth-weight infants, the number of readmissions because of episodes of acute premature labor, or any other neonatal outcome evaluated (Table III).

Other outcomes (Table III) of clinical interest were not different but require explanation. In addition to the three women who were diagnosed with clinical cho-

^{*}Recovery or identification rates were not different, except as noted.

[†]Not all microbial samples were evaluable and not all subjects were available for repeat examination.

p < 0.05.

p < 0.01.

^{*}Mean ± SD. Range is shown in parentheses.

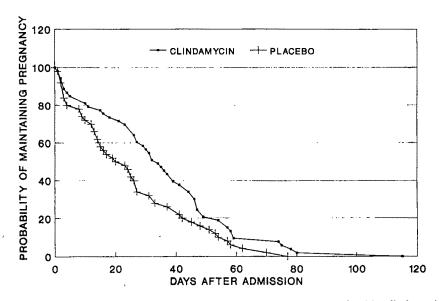


Fig. 1. Survival analysis for continuing pregnancy in women treated with clindamycin or placebo. Clindamycin treatment was associated with significantly prolonged pregnancy (see text).

Table IV. Summary table of multiple regression analysis predicting days from study entry to delivery

Variable	Regression coefficients	Significance
Clindamycin treatment	-3.95	0.03
Gestational age at enrollment	-1.93	0.008
Cervical dilatation at enrollment	-9.37	0.0001
Cervical effacement at enrollment	-3.38	0.09

$$R^2 = 0.39$$
; F = 15.68; $p = 0.0001$.

rioamnionitis after a single dose of study treatment and who were excluded from analysis, clinical chorioamnionitis later developed in one clindamycin patient and three placebo patients. Four women in each treatment group were treated for postpartum endometritis. Two newborns (one from each group) were treated for bacteremia and four were treated for pneumonitis. Two neonatal deaths occurred, each of the mothers of these infants had received clindamycin. One infant who died was born at 25 weeks' gestation and weighed 815 gm; the other was 27 weeks' gestation at birth and weighed 940 gm. Both deaths were attributed to complications of prematurity. Two additional infant deaths were attributed to sudden infant death syndrome; one from the clindamycin group occurred at age 31/2 months inan infant born at 35 weeks' gestation, and one from the placebo group occurred at age 7 months in an infant born at 32 weeks' gestation.

To further explore the role of microorganisms in preterm birth, we compared women with and without specific microorganisms or conditions and controlled for study treatment. Overall, 25% of enrolled women had bacterial vaginosis; 20 of these 25 women (80%) were delivered preterm. In comparison, 41 of 74 women (55.4%) without bacterial vaginosis were delivered preterm (p = 0.03; relative risk, 1.4; 95% confidence interval, 1.04 to 2.0). Logistic regression analysis confirmed that bacterial vaginosis was the only microbiologic independent variable that predicted preterm birth (p < 0.05; odds ratio, 3.2; 95%, confidence interval 1.1 to 9.6). Clindamycin treatment in women with bacterial vaginosis showed trends for prolongation of pregnancy (clindamycin-treated group, 35.7 days gained; placebo-treated group, 19.0 days gained; p =0.07) and increased infant birth weight (clindamycintreated group, 2634 gm; placebo-treated group, 2296 gm; p = 0.1). There was a corresponding but statistically insignificant increase in gestational age at delivery (clindamycin-treated group, 35 weeks, placebo-treated group, 34 weeks; p = 0.16) and a corresponding decrease of low-birth-weight infants (clindamycin-treated group, 3/15 [20%]; placebo-treated group, 5/10 [50%]; p = 0.19) among clindamycin-treated women with bacterial vaginosis. Preterm birth occurred among 11 of 15 clindamycin-treated women (73.3%) who were initially found to have bacterial vaginosis versus 9 of 10 among similar placebo-treated women (90%).

Recovery of *M. hominis, U. urealyticum,* or *G. vaginalis* was not associated with prematurity or low birth weight. No treatment-associated differences in outcome variables were detected when presence of these microorganisms was controlled. Potential impact on studied pregnancy outcomes associated with the presence of group B streptococcus, *C. trachomatis, T. vaginalis,* and

S. aureus were not evaluated individually because of the small numbers of women from whom these microorganisms were identified.

To further define mother-perinate pairs who possibly benefited from clindamycin treatment, outcomes were categorized for women with microbes (other than bacterial vaginosis-associated microorganisms) such as group B streptococcus, C. trachomatis, T. vaginalis, or S. aureus that were previously implicated in premature birth. Each of these microorganisms, except for T. vaginalis, is susceptible to clindamycin. This subset of women with either group B streptococcus, C. trachomatis, T. vaginalis, or S. aureus was more likely to have preterm premature rupture of membranes after study entry than were women without these microorganisms (positive for microbes, 5/17 [30%]; negative for microbes, 7/86 [8%]; p = 0.01). Clindamycin treatment of these infected women reduced the incidence of preterm premature rupture of membranes to that of uninfected subjects (clindamycin-treated group, positive for microbes 1/9 [11%] and microbe negative for 4/44 [9%]; placebo-treated group, positive for microbes 4/8 [50%] and negative for microbes 3/42 [7%]). As noted, study outcomes did not correlate with the presence of amniotic fluid white blood cells among women undergoing amniocentesis (53%) either when considered alone or when study treatment was con-

Side effects were reported among 28% of clindamycin-treated women and 32% of placebo-treated women. Nausea and vomiting were the most common side effects, they occurred among 17% and 28% of clindamycin- and placebo-treated patients, respectively. Diarrhea (loose stools four to five times a day) was reported in five women (9.4%) who received clindamycin and in three women (6.0%) who received placebo. None of these women required discontinuation of treatment; no patient experienced pseudomembranous enterocolitis. Clostridium difficile toxin production was not detected. Two patients treated with clindamycin and one placebo-treated patient received treatment for yeast vulvovaginitis after study enrollment.

Comment

In this controlled trial we evaluated the efficacy, safety, and tolerance of a defined course of clindamycin versus placebo in prolonging pregnancy in hospitalized pregnant women treated with parenteral tocolysis ≤34 weeks' gestation. To further probe the effects of possible reproductive tract infection in mediating preterm birth, we also sought to identify obstetric and/or microbiologic parameters that identified women who benefited from clindamycin treatment. Pregnancies continued approximately 10 days longer in clindamycintreated women than in women receiving placebo (p = 0.02). Newborns of treated women required fewer days of level II or III nursing care (7.1 vs 16.8 days). Bacterial vaginosis was associated with a greater risk of preterm delivery. Women with group B streptococcus, C. trachomatis, T. vaginalis, or S. aureus were more likely to have subsequent preterm premature rupture of membranes. Clindamycin treatment was associated with reduced risk of preterm premature rupture of membranes in these women. Stratification for gestational age at enrollment showed that clindamycin was associated with a significantly prolonged interval to delivery only among women enrolled ≤32 weeks' gestation. Treatment appeared safe and well tolerated.

Limitations of this study include the number of subjects studied (103) and the inherent inability to precisely measure outcome variables, such as gestational age. End points, such as days to delivery or days of intensive nursery care, were more accurately determined than were clinically derived estimates of gestational length. Given the presumed heterogeneity of factors that cause preterm birth, many of which would not be affected by clindamycin treatment, a sample of 100 patients may be inadequate to detect other clinically relevant differences. For example, a sample of 66 women with bacterial vaginosis would be required to detect, with 80% probability, a significant increase in birth weight, with the assumption that the trend noted in this study was a true difference.

In spite of study limitations our finding of a 10-day overall prolongation of pregnancy associated with clindamycin treatment offers important benefits. In a large epidemiologic study Goldenberg et al.14 calculated that an infant's chance of surviving without major handicap increased up to 3% for each day of continued pregnancy between 23 and 30 weeks' gestation. Korenbrot et al.,15 who calculated direct costs, estimated that prolongation of pregnancy for women between 26 and 33 weeks' gestation resulted in savings of \$11,240 (in 1981) per birth. Hernandez et al.16 derived similar calculations in a Denver population. Total "costs" for preterm birth, including suffering and lost human potential for individual newborns, their families, and society, remain incalculable.

The microbiologic findings in this study confirm suggestions that bacterial vaginosis plays important roles in the pathogenesis of preterm birth. 17-20 Gravett et al. 17 reported findings of bacterial vaginosis among 43% of patients hospitalized with preterm labor versus 14% of matched control subjects without preterm labor. Subsequently, Martius et al.19 reported bacterial vaginosis in 55.7% of women with preterm labor who were delivered preterm compared with 15.6% of women who were delivered at term without preterm labor. In our study, preterm birth also occurred more frequently in the presence of bacterial vaginosis (p = 0.03). Newborns of clindamycin-treated women with bacterial vaginosis weighed a mean of 340 gm more than did infants of placebo-treated women. Failure of this dramatic difference to reach statistical significance should not overshadow the potential clinical benefits associated with prolongation of pregnancy. The relatively small number of women with bacterial vaginosis studied probably accounts for the lack of statistical significance.

Comparison of these findings with reported controlled trials of antibiotics among women in preterm labor suggests that responses differ in the various experimental antibiotic treatments and that factors mediating preterm birth probably vary among populations.⁸⁻⁶ We previously conducted a double-blind, randomized, placebo-controlled trial of oral erythromycin that demonstrated prolongation of pregnancy only among women with cervical dilatation ≥1 but ≤4 cm.3 In a similar study, Winkler et al.4 duplicated these findings and suggested that benefits occurred only among women with genital U. urealyticum. Subsequently, Morales et al.5 administered either ampicillin or erythromycin to a larger group of women and noted prolongation of pregnancy for each treatment group compared with untreated controls. They reported that only ampicillin treatment was associated with prolongation of pregnancy among G. vaginalis-colonized women, although the use of either antibiotic was associated with prolonged pregnancy in women with group B streptococcus but without G. vaginalis. Neither study specifically evaluated bacterial vaginosis or defined microbial findings after treatment.

In contrast, Newton et al.6 studied a predominantly Hispanic population and noted no benefit associated with intravenous ampicillin administered in addition to oral erythromycin.6 Bacterial vaginosis was common (41%). There was neither a reduction of these findings subsequent to treatment nor any improvement of pregnancy outcomes within this large subgroup. This suggests that effective treatment of bacterial vaginosis is linked to successful prolongation of pregnancy in women treated for preterm labor. Associations between group B streptococcus or other microbiologic findings and preterm birth were not discussed. In addition, fewer women in their study were delivered preterm (44% overall in the study by Newton et al.6 69% in the study by Morales et al.,5 and 62% in our study); this may have reduced the likelihood of detecting treatment effects.

In our study clindamycin treatment was associated with reduced identification of bacterial vaginosis and with the recovery of *M. hominis* but not *U. urealyticum*. In a prior non-treatment-based prospective study of microbiologic agents and complications of pregnancy, we noted an association between recovery of *M. hominis*

from the genital tract and bacterial vaginosis in women who subsequently had preterm labor. Recently, clindamycin has been shown to be an effective treatment for bacterial vaginosis. In our study clindamycin treatment remained in the multivariate regression model (p=0.03) and was associated with prolongation of pregnancy, regardless of cervicovaginal microbiologic findings. This suggests that clindamycin is effective against susceptible microorganisms that cause intrauterine infection (in the decidua or amniochorion) although not necessarily associated with the specific lower genital tract findings sought in our study.

Use of a defined course of intravenous (3 days) and subsequent oral (4 days) clindamycin may offer relative advantages in comparison with use of erythromycin and/or ampicillin. Use of oral medications among patients in labor may be associated with inadequate absorption that results from a delay in gastric emptying or vomiting, especially in women treated with tocolytics. The pharmacokinetics of clindamycin during pregnancy are well studied. Clindamycin also provides relatively focused antimicrobial coverage for agents previously implicated in preterm birth, including bacterial vaginosis, group B streptococcus, C. trachomatis, and genital mycoplasmas (especially M. hominis), as well as a broad variety of anaerobic bacteria. 17-20

Other features of clindamycin use also may offer relative advantages in the treatment of preterm labor. Unlike erythromycin, clindamycin is functionally active at pH conditions common in the vagina and readily crosses the placental barriers to achieve therapeutic concentrations within gestational tissues and amniotic fluid.23, 24 In contrast to ampicillin, clindamycin is concentrated relative to maternal serum within the amnion and chorion.25 This may prove important, because Hillier et al.20 showed that microorganisms were recovered between layers of amniochorion in more than half of women who were delivered before 32 weeks' gestation.20 Clindamycin also may nonspecifically enhance maternal-host defenses. Clindamycin penetrates into polymorphonuclear leukocytes, increases intercellular killing or inhibition of bacteria, and reduces adherence among various microorganisms.26 Such features suggest that clindamycin or similar agents may provide selective advantages and benefits in treatment of instances of preterm labor attributable to reproductive tract infection. Conversely, the use of β-lactam antibiotics, such as ampicillin, may hasten processes involved in preterm labor. These agents effect bacterial cell lysis, with subsequent release of bacterial contents, and may foster further tissue damage. Use of antibiotics such as clindamycin and ampicillin has been associated with antibiotic-induced diarrhea, including C. difficile toxin-associated pseudomembranous enterocolitis.

In our study treatment with an adjunctive-defined

course of clindamycin was associated with significant prolongation of pregnancy in hospitalized women treated for preterm labor. This effect appeared to be limited to women enrolled at ≤32 weeks' gestation. Additional studies are required to confirm these findings and to refine indications for appropriate antimicrobial treatment for women at risk for preterm birth. Studies to evaluate whether significant numbers of preterm births can be prevented by effective treatment of reproductive tract infection either early in pregnancy or before pregnancy are ongoing.

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Fetal cardiac function in intrauterine growth retardation

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Blood-flow velocity waveforms are altered in several peripheral vascular beds of fetuses whose intrauterine growth is retarded because of placental insufficiency. We investigated these concomitant changes in cardiac function. Color and pulsed Doppler echocardiographic recordings were performed in 124 fetuses with intrauterine growth retardation. These fetuses were free of structural and chromosomal abnormalities and were characterized by increased umbilical artery resistance and reduced middle cerebral artery resistance. Twenty-four of these fetuses were also studied at weekly intervals until the onset of antepartum late heart rate decelerations. Blood-flow velocity waveforms were obtained from the aortic and pulmonary valves, and the following variables were measured: peak systolic velocity, time to peak velocity, the product of time velocity integral multiplied by heart rate, left and right cardiac output, and the right/left ratios of the product of time velocity integrals multiplied by heart rate and cardiac output. When compared with previously established norms, both aortic and pulmonary peak systolic velocities and pulmonary time to peak velocity were reduced; aortic time to peak velocity increased. Left cardiac output and the product of the aortic time velocity integral multiplied by the heart rate increased and right cardiac output and the product of the pulmonary time velocity integral multiplied by the heart rate decreased, resulting in reduced right/left ratios. In the 24 fetuses studied longitudinally, time to peak velocities and the right/left flow ratios remained stable. However, aortic and pulmonary peak velocities and cardiac output declined significantly in contrast to an expected rise with advancing gestation. The fall in cardiac output and aortic and pulmonary peak velocities was directly related to umbilical artery pH at birth. This study provides evidence of a modified cardiac function that seems to deteriorate progressively with the advancing gestation of fetuses with intrauterine growth retardation. (AM J OBSTET GYNECOL 1991;165:876-82.)

Key words: Intrauterine growth retardation, Doppler ultrasonography, fetal heart, echocardiography

Intrauterine growth retardation (IUGR) caused by placental insufficiency is associated with altered Doppler blood flow velocity waveforms in several vascular beds, including the umbilical artery, descending aorta, renal artery, and cerebral vasculature. These changes suggest selective modification of peripheral vascular resistance, which leads to preferential perfusion of the brain with respect to the lower body (i.e., the so-called brain-sparing condition).

Little study has been done on cardiac function in IUGR, and the findings obtained are controversial. Reed et al.⁵ observed increased right cardiac output, and we⁶ and Al Ghazali et al.⁷ observed a redistribution of cardiac flow in favor of the left side of the heart. These earlier studies used different criteria for the selection of fetuses, and their sample sizes were relatively small. The purpose of our investigation was twofold:

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first, to elucidate the changes of cardiac function present in IUGR caused by placental insufficiency; and second, to discover the possible further modifications of cardiac function from the time IUGR is diagnosed to the onset of antepartum late heart rate decelerations.

Material and methods

Subjects. After we obtained informed consent from the mothers, 124 fetuses with IUGR were evaluated (Table I). Entry criteria were (1) singleton gestation accurately dated by early second-trimester fetal biparietal diameter measurement; (2) abdominal circumference <5th percentile of our reference limits (fetuses with a gestational age <28 weeks) or ultrasonographically estimated fetal weight (fetuses with a gestational age ≥28 weeks) <5th percentile for our population; (3) absence of congenital or chromosomal abnormalities; and (4) abnormal blood flow velocity waveforms suggesting brain sparing (i.e., ratio of umbilical artery/middle cerebral artery pulsatility index >95th percentile for our reference limits).8 The diagnosis of IUGR (i.e., birth weight <5th percentile in a newborn free of structural abnormalities) was postnatally confirmed in all subjects.

It was also possible to study longitudinally a subgroup of these pregnancies at weekly intervals from the diagnosis of IUGR until delivery. To obtain a homoge-

neous group, we retained only the data of 24 fetuses who had antepartum late heart rate decelerations and who had been studied on at least four occasions I week apart (longitudinal study) (Table I). Fetal heart rate monitoring was performed daily for ≥45 minutes from the time of diagnosis. At the onset of late heart rate decelerations during Braxton Hicks contractions, elective cesarean sections were performed. For control, 26 healthy fetuses were similarly studied at weekly intervals from 27 to 33 weeks' gestation.

Umbilical artery pH was measured at delivery in 94 fetuses with IUGR (21 belonging to the longitudinal subgroup) on double-clamped segments of the cord. All measurements were performed ≤5 minutes from delivery with an automated blood gas analyzer (ABL 30, Radiometer A/S, Copenhagen).

Doppler echocardiographic examination. Commercially available color Doppler echocardiographic equipment (Esacord 81, Ansaldo Hitachi, Genoa) with a 3.5 or 5 MHz convex probe was used for all measurements. In addition to the color flow mapping function, the machine was equipped with a pulsed Doppler instrument with a carrier frequency that ranged from 2.5 to

Velocity waveforms were recorded in the ascending aorta and pulmonary artery according to a previously reported technique.9 The aortic valve was visualized on the five-chamber view, and the pulmonary valve was visualized on the short-axis view. The color flow mapping function was superimposed and the valve flow measured. Care was taken to visualize flow so that it was parallel to the Doppler echocardiographic beam. The sample site was just distal to the valve leaflets. Recordings obtained with a beam angle >20 degrees were rejected.

Doppler echocardiographic and real-time images were recorded on standard half-inch videotape for subsequent analysis. Valve diameter measurements were made at playback of videorecorded images frozen during diastole at the tip of the closed arterial valve. Measurements were performed at 10 different cycles for each valve, and the values were averaged. Valve area was calculated by assuming a circular cross section.

Permanent records of the velocity waveforms were made on a strip chart recorder from the videotape. Ten consecutive velocity waveforms were selected during periods of fetal rest without breathing movements, and the values were averaged (Fig. 1). The following variables were measured with the aid of a computer-interfaced digitizer pad (Cardio 800, Kontron Instruments, Oxford, England): (1) peak velocities during systole; (2) time to peak velocity, calculated as the time difference from the onset of the waveform to its peak velocity⁷; (3) time velocity integral; and (4) heart

The absolute right and left cardiac output (milliliters

Table I. Characteristics of the fetuses with IUGR studied

All subjects	
Gestational age at echocardiographic recording (wk, mean ± SD)	30.4 ± 2.8
Umbilical artery pulsatility index (mean ± SD)	1.74 ± 0.32
Middle cerebral artery pulsatility in- dex (mean ± SD)	1.24 ± 0.27
Gestational age at delivery (wk, mean ± SD)	33.7 ± 3.6
No. of intrauterine deaths (%)	3 (2.4)
No. of emergency cesarean sections for fetal distress (%)	84 (67.7)
Birth weight (gm, mean ± SD)	1589 ± 457
Umbilical artery pH at delivery (94 cases, mean ± SD)	7.120 ± 0.076
No. of admissions to neonatal intensive care unit (%)	93 (75)
Longitudinal study (subgroup, 24 cases)	
Umbilical artery pulsatility index (first recording, mean ± SD)	1.76 ± 0.24
Umbilical artery pulsatility index (last recording, mean \pm SD)	1.94 ± 0.33
Middle cerebral artery pulsatility in- dex (first recording, mean ± SD)	1.32 ± 0.19
Middle cerebral artery pulsatility in- dex (last recording, mean ± SD)	1.21 ± 0.26
Gestational age at echocardiographic recording (wk, mean ± SD)	26.6 ± 1.5
Gestational age at delivery (wk, mean ± SD)	32.8 ± 2.4
Interval between last echocardiogra- phic recording and delivery (days, mean ± SD)	2.45 ± 1.89
Birth weight (gm, mean ± SD)	1235 ± 247
Umbilical artery pH (21 cases, mean ± SD)	7.118 ± 0.051
No. admitted to neonatal intensive care unit (%)	24 (100)

per minute) was calculated by multiplying the pulmonary or aortic time velocity integral, valve area, and heart rate. Because valve area calculations have a relatively high coefficient of variation,7.10 cardiac flow was also expressed as the product of time velocity integral multiplied by heart rate, an index related to absolute cardiac output.11 Last, the combined cardiac output (left cardiac output plus right cardiac output) and the ratios between right cardiac output and left cardiac output and between the pulmonary time velocity integral multiplied by heart rate and aortic time velocity integral multiplied by heart rate were calculated.

To correct cardiac output for fetal size, the combined cardiac output was corrected for fetal weight and expressed as milliliters per minute per kilogram. However, because the ultrasonographic estimation of fetal weight may add a source of error to the difficult calculation of cardiac output, this analysis was limited to the last recording performed in the fetuses with IUGR who were studied longitudinally. In this instance we used the neonatal weight.

Data analysis. The variables measured were com-

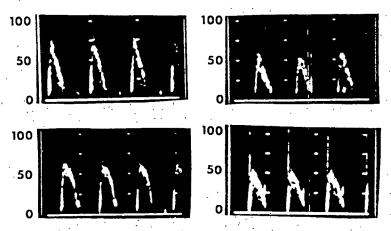


Fig. 1. Flow velocity waveforms recorded at 34 weeks' gestation at aortic valve level (top panel) in normal fetus (right: peak velocity = 77 cm/sec, time to peak velocity = 43 msec) and fetus with IUGR (left: peak velocity = 58 cm/sec, time to peak velocity = 61 msec) and at pulmonary valve level (bottom panel) in normal fetuses (right: peak velocity = 61 cm/sec, time to peak velocity = 38 msec) and fetus with IUGR (left: peak velocity = 51 cm/sec, time to peak velocity = 21 msec). Values are in centimeters per second.

pared with our reference population of 197 normal fetuses between 20 and 40 weeks' gestation. ^{10, 12} Differences were evaluated by means of one sample and unpaired Student's t tests. The longitudinal changes in echocardiographic parameters were evaluated by the analysis of variance for repeated measurements. Simple and multiple regression analyses were used to test the relationships between the variables considered. Differences yielding $p \le 0.05$ were considered significant.

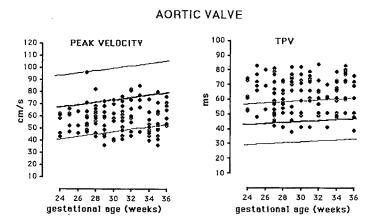
Results

Cross-sectional study. Successful recordings were obtained in 121 (97.6%) fetuses at the aortic valve level and in 116 (93.5%) at the pulmonary valve level. Peak velocity values recorded at the aortic (t = 8.341, $p \le 0.001$) and pulmonary ($t = 8.973, p \le 0.001$) valve levels for fetuses with IUGR were significantly lower than for our reference populations (Fig. 1). Aortic time to peak velocity was higher (t = 14.779, $p \le 0.001$) and pulmonary time to peak velocity lower (t = 9.546, $p \le 0.001$) than normal in fetuses with IUGR (Fig. 2). Aortic time velocity integral multiplied by heart rate $(t = 2.643, p \le 0.01)$ and left cardiac output $(t = 2.348, p \le 0.01)$ $p \le 0.02$) were significantly higher in IUGR compared with control fetuses. Pulmonary time velocity integral multiplied by heart rate ($t = 1.989, p \le 0.05$) and right cardiac output (t = 2.371, $p \le 0.02$) were lower in fetuses with IUGR. No significant changes were found in combined cardiac output (t = 0.881, not significant [NS]). A consequence of the increased left flow associated with the decreased right flow was that the right cardiac output/left cardiac output (t = 18.947, $p \le$ 0.001) and pulmonary time velocity integral multiplied by heart rate/aortic time velocity integral multiplied by

heart rate (t = 15.729, $p \le 0.001$) ratios were reduced in IUGR (Fig. 3).

Longitudinal study. Pregnancy outcome and delivery were uneventful for the control fetuses. Their gestational age (mean \pm SD) at delivery was 40.3 ± 1.8 weeks; birth weight was 3345 ± 435 gm. In these fetuses the pulsatility index from the umbilical artery decreased with advancing gestation (F = 7.123, $p \le 0.001$), and the pulsatility index of the middle cerebral artery was unchanged (F = 1.212, NS). As a result, the ratio between the umbilical artery and the middle cerebral artery pulsatility index significantly decreased (F = 2.435, $p \le 0.005$). At the intracardiac level, peak velocity at the aortic (F = 2.012; $p \le 0.01$) and pulmonary (F = 1.987, $p \le 0.01$) valve levels rose progressively with advancing gestation. Similarly left cardiac output (F = 18.639, $p \le 0.001$), right cardiac output (18.457, $p \le 0.001$), aortic time velocity integral multiplied by heart rate (F = 2.194, $p \le 0.005$), and pulmonary time velocity integral multiplied by heart rate (F = 2.077, $p \le 0.01$) rose with gestational age. Over the interval studied, no significant alterations in aortic time to peak velocity (F = 0.463, NS), pulmonary time to peak velocity (F = 0.784, NS), right cardiac output/left cardiac output (F = 0.521, NS), and pulmonary time velocity integral multiplied by heart rate/aortic time velocity integral multiplied by heart rate ratio (F = 0.437, NS) were seen.

IUGR fetuses showed a significant increase in the umbilical artery pulsatility index (F = 2.022, $p \le 0.01$) and a significant decrease in the middle cerebral artery pulsatility index (F = 1.931; $p \le 0.01$). Therefore the ratio between the umbilical artery and middle cerebral artery increased further (F = 2.424, $p \le 0.005$). Peak



PULMONARY VALVE .

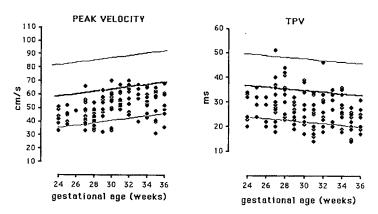


Fig. 2. Aortic and pulmonary peak velocity and time to peak velocity (TPV) values in fetuses with IUGR plotted on our reference ranges for gestation (mean and 95% confidence intervals).

velocity values at both the aortic (F = 11.423, $p \le 0.001$) and pulmonary (F = 9.358, $p \le 0.001$) valve levels declined progressively in fetuses with IUGR (Fig. 4). Furthermore, the left cardiac output (F = 8.267, $p \le 0.001$), right cardiac output (F = 7.975, $p \le 0.001$), combined cardiac output (F = 14.676, $p \le 0.001$), aortic time velocity integral multiplied by heart rate (F = 12.358, $p \le 0.001$), and pulmonary time velocity integral multiplied by heart rate (F = 10.755, $p \le 0.001$) decreased significantly with advancing gestation. No significant variations were found in aortic (F = 0.699, NS) and pulmonary (F = 0.730, NS) time to peak velocity values, and in right cardiac output/left cardiac output (F = 0.586, NS) and pulmonary time velocity integral multiplied by heart rate/aortic time velocity integral multiplied by heart rate ratios (F = 0.974, NS). The individual changes of aortic peak velocity, pulmonary peak velocity, and combined cardiac output found in IUGR are plotted in Fig. 4. When the combined cardiac output of fetuses with IUGR obtained at the last recording was corrected for neonatal weight and compared with the

control group, a statistically significant difference was proved (IUGR combined cardiac output = 395 ± 124 ml/min/kg; control combined cardiac output = $525 \pm 96 \text{ ml/min/kg}$; t = 6.05; $p \le 0.001$). The echocardiographic parameters obtained during the last recording were corrected for gestational age (i.e., number of standard deviations by which they differ from the expected mean of gestation) and related to umbilical artery pH at birth. A positive correlation was found between pH and peak velocity in ascending aorta $(r = 0.43; p \le 0.05)$, peak velocity in pulmonary artery (r = 0.51; $p \le 0.03$), and combined cardiac output values (r = 0.67; $p \le 0.01$) with a multiple correlation coefficient of 0.71. No significant relationships were noted between the severity of IUGR (as evident in the birth weight percentile) and the Doppler echocardiographic examination variables considered.

Comment

During fetal life, the output of the left ventricle is directed through the ascending aorta mainly to the brain, whereas the output of the right ventricle is di880 Rizzo and Arduini

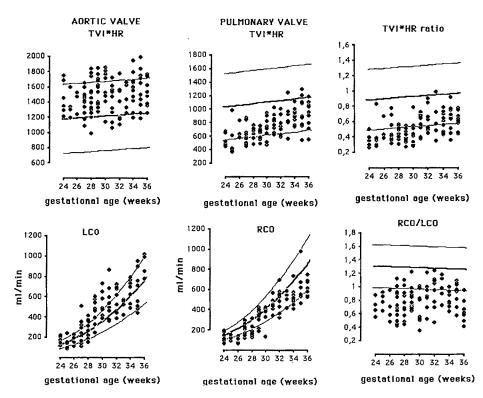


Fig. 3. Products of time velocity integral multiplied by heart rate (TVI*HR) in aortic and pulmonary valves, left (LCO) and right (RCO) cardiac output, right/left ratio of products of time velocity integrals multiplied by heart rate (TVI*HR ratio) and ratio of cardiac output (RCO/LCO) in fetuses with IUGR plotted on our reference ranges for gestation (mean and 95% confidence intervals).

rected through the ductus arteriosus to the lower body and placenta. Previous Doppler echocardiographic studies and the present longitudinal data have shown that in normal fetuses, peak flow velocity rises with gestation at both the aortic and pulmonary valves. These changes are probably caused by several factors, including a progressive improvement in myocardial contractility, a reduction of cardiac afterload, and an increase in cardiac preload. 13, 14 Study of the time to peak velocity (a parameter inversely related to blood pressure) suggests that in healthy fetuses, the mean pressure is slightly higher in the pulmonary artery than in the ascending aorta.15 Finally, it has been shown that cardiac output increases progressively with gestation and that right cardiac output is slightly higher than left cardiac output, with a right cardiac output/left cardiac output ratio of about 1.3.13, 16

Doppler echocardiographically measured cerebral vascular resistance in fetuses whose IUGR is caused by placental insufficiency is lower than normal and the placental resistance is higher than normal, suggesting that there is a selective change in cardiac afterload (i.e., decreased left ventricular and increased right ventricular afterload). IUGR caused by placental insufficiency is frequently associated with both hypoxemia, which may impair cardiac contractility, 7 and polycythemia, 18

which could alter viscosity and therefore cardiac preload.

Both the selection criteria and the pregnancy outcome strongly suggest that the growth defects of the fetuses in this study resulted from placental insufficiency. These fetuses with IUGR showed reduced peak velocity, increased aortic and decreased pulmonary time to peak velocity, and a relative increase of left cardiac flow associated with decreased right cardiac flow. These data confirm previous results in smaller series of fetuses with IUGR.^{6, 7, 9, 19}

The parameters measured in this study are influenced by various factors such as preload, afterload, intrinsic contractile properties of the left and right ventricles, and valve dimensions. The noninvasive nature of the human fetal model does not allow further differentiation between these factors.

There has been debate as to whether time to peak velocity values in the human fetus reflect mainly the pressure in the aorta and the pulmonary artery, and therefore the impedance and capacitance of the distal vascular districts, or whether they are also influenced by the intrinsic contractile properties and preload conditions of the two ventricles. ^{15, 20} The lack of data on simultaneous measurements of flow and pressure does not allow us to differentiate between these explana-

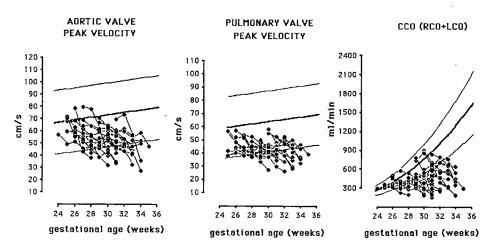


Fig. 4. Individual changes in aortic and pulmonary peak velocity and in combined cardiac output (CCO = left plus right cardiac output) values in the 24 fetuses with IUGR studied longitudinally plotted on our reference ranges for gestation (mean and 95% confidence intervals). Values of each patient are connected by lines.

tions. However, the inverse modifications of pulmonary and aortic time to peak velocity suggest the presence of different functional conditions between the right and left ventricles in fetuses with IUGR. This justifies the preferential shift of cardiac output in favor of the left ventricle, leading to improved perfusion to the brain. Thus the supply of substrate and oxygen could be maintained at near-normal levels in spite of any absolute reduction of placental transfer. The reduced peak velocity values could have multiple causes, including decreased myocardial contractility, modifications of preload, pressure, volume flows, and valve dimension.

Doppler echocardiographically derived estimates of hemodynamic parameters in the human fetus must be interpreted with caution because absolute calculations of volume flows are prone to error resulting from the inaccuracies in determining valve diameters (which are then halved and squared in the calculation). However, we sought to minimize this bias by eliminating the measurement of valve area from the calculation (time velocity integral multiplied by heart rate). The results were unaltered and thus supported the reliability of our findings.

Contradictory findings were reported by Reed et al.,5 who observed increased flow in the right side of the heart in a study of 12 fetuses selected on the basis of absent umbilical artery end-diastolic velocities. However, four fetuses in this series had chromosomal or structural abnormalities and four showed either pericardial or pleural effusions, suggesting that many of the fetuses studied had IUGR that was not solely the result of placental insufficiency. This conclusion is supported by previous Doppler echocardiographic studies that showed the absence of brain sparing in fetuses with IUGR who also had structural or chromosomal abnormalities in spite of abnormalities in umbilical artery velocity waveforms.21.22 A difference in the adaptive vascular mechanism might explain the discrepancy in the cardiac studies.

In both the cross-sectional evaluation and the first recording of the longitudinal study, fetuses with IUGR showed combined cardiac output values similar to those of normal fetuses of corresponding gestational age, implying relatively higher output with respect to fetal weight. These findings confirm a prior report and suggest that in a first step of the disease (the interval between the Doppler echocardiographic recordings and delivery in the cross-sectional study was >3 weeks [range 1 to 7 weeks]) the cardiac output is not only diverted toward the left side but also is relatively in-

Our longitudinal study of fetuses with IUGR allowed us to elucidate the natural history of cardiovascular modifications. Because the time to peak velocity and the ratios between right and left ventricular flows remained stable, it appears that there are no other significant changes in outflow resistance and cardiac output redistribution after the establishment of the brainsparing mechanism in spite of the further worsening of the ratio between the umbilical artery and the middle cerebral artery pulsatility index. However, it is important to note that in IUGR peak velocity and cardiac output progressively declined rather than rising with gestation as expected, and the fall of combined cardiac output values obtained at the last recording were also significantly lower after correction for weight. Similarly, studies in animals showed a significant reduction of cardiac output during chronic preparations designed to retard fetal growth.23 A significant correlation has been found between the reduction of peak velocity in the pulmonary artery and the severity of placental infarction in the human fetus.¹⁹

The physiologic significance of these longitudinal changes is subject to several interpretations. Because they are closely followed by the onset of abnormal heart rate patterns and are related to umbilical artery pH at birth, we speculate that the fall in cardiac output and in peak velocity terminally may be related to a progressive compromise of fetal oxygenation and acid-base status.

In conclusion, abnormalities of cardiac function are present in fetuses with IUGR caused by placental insufficiency; they differ between the right and left ventricles. In fetuses studied longitudinally until the onset of spontaneous late heart rate decelerations a further decrease of cardiac output and pulmonary and aortic peak velocities was found.

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When do cardiovascular parameters return to their preconception values?

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To determine if the postpartum period is reflective of a woman's cardiovascular status before pregnancy, we performed serial studies of 13 women before conception and at 6 and 12 weeks post partum. All pregnancies were singleton without hypertensive complications. Cardiac output, stroke volume, and end-diastolic volume were calculated with M-mode echocardiography from the left ventricular dimensions with subjects in the left lateral position. Systemic vascular resistance was calculated from cardiac output and simultaneous measurements of blood pressure. Stroke volume and end-diastolic volume remained consistently elevated over preconception values at 6 and 12 weeks. Systemic vascular resistance remained decreased, compared with baseline, at 12 weeks. Thus cardiovascular parameters had not returned to the preconception baseline, and previous studies that have used this time period for comparison have underestimated the contribution of stroke volume to the total change in cardiac output during pregnancy. (AM J OBSTET GYNECOL 1991;165:883-6.)

Key words: Maternal echocardiography, pregnancy, postpartum period, cardiovascular

The cardiovascular system undergoes profound alterations during pregnancy. Increases in heart rate, stroke volume, end-diastolic volume, and cardiac output and decreases in mean arterial pressure and systemic vascular resistance have been well documented with several different techniques. ¹⁻³ However, previous studies may have underestimated the magnitude of the cardiovascular changes in pregnancy, because baseline measurements were not obtained before pregnancy but rather were compared with postpartum values. It has not been established when or whether cardiovascular function returns to its preconception state. This study was undertaken to address this issue.

We have previously reported on the cardiovascular changes in early pregnancy determined with M-mode echocardiography. When subjects were studied before pregnancy and serially during pregnancy, significant increases in stroke volume, left ventricular end-diastolic volume, and cardiac output could be documented in the first trimester. Robson et al., with Doppler and M-mode echocardiography, have documented similar changes when subjects were initially studied before pregnancy and serially during pregnancy. This report extends our earlier studies to include the postpartum

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period. We hypothesized that the central cardiovascular changes that occur in pregnancy do not return to their baseline state by 6 weeks post partum. A longitudinal serial study design in which each subject was studied before conception was used so that comparisons could be based on each subject's preconception baseline.

Material and methods

Thirteen subjects were initially evaluated within 3 months of conception. All subjects were nonsmoking, physically active women with no history of heart disease, and all were delivered of singleton infants after 37 weeks without hypertensive complications. All women were breast-feeding when studied at 6 and 12 weeks post partum. The mean age was 30.6 years, with a range of 25 to 36 years. There were seven primiparous and six multiparous women.

All studies were preceded by an initial 15-minute accommodation to the laboratory environment. At this time, a heart rate monitor and blood pressure cuff were placed, and the subject assumed a comfortable left lateral position. Blood pressure was measured every 2 minutes with a mercury sphygomomanometer that was zeroed at the level of the subject's left ventricle. M-mode echocardiography was performed with either a GE 3600 or an ADR Ultramark 4 ultrasonography machine. The left parasternal window was used to obtain the long-axis view of the heart, which visualized the left ventricle and the mitral and aortic valves with real-time scanning. The M-mode beam was then positioned just below the tips of the mitral valve leaflets. Measurements of end-systolic and end-diastolic dimensions were made in accordance with the guidelines published by the

Table I. Cardiovascular measurements

	Before pregnancy	6 wk post partum	12 wk post partum
End-diastolic volume (ml)	107 ± 6	124.1 ± 7*	119 ± 5*
Stroke volume (ml)	68 ± 3	81 ± 4*	79 ± 3*
Cardiac output (L/min)	4.3 ± 0.2	4.6 ± 2	$4.9 \pm 0.2\dagger$
Heart rate (beats/min)	64 ± 2	57 ± 3	62 ± 3
Mean arterial pressure (mm Hg)	70 ± 2	74 ± 3	69 ± 3
Systemic vascular resistance (dynes × sec × cm ⁻⁵)	1349 ± 83	1277 ± 65	1154 ± 70*

Values are mean ± SEM.

American Society of Echocardiography.⁶ An additional 5 minutes of imaging preceded the initial measurements in an effort to further accommodate the subject to the study. Heart rate was directly measured during the intervals in which cardiac output was determined. Three or more separate sets of measurements were obtained over a 15-minute period. Thus the values obtained represent the average of 27 to 50 separate cardiac cycles interspersed throughout the respiratory cycle.

Left ventricular volumes were calculated from the end-diastolic and end-systolic dimensions in accordance with the formula of Teichholz et al. for a modified prolate ellipsoid. These data were then used to calculate stroke volume, cardiac output, mean arterial pressure, and systemic vascular resistance with standard formulas.

The calculations of stroke volume with the Teichholz equation has been validated against angiography-derived stroke volume with a correlation coefficient of r=0.97.7 When the technique was compared with thermodilution cardiac output, the correlation coefficient was r=0.86.8 Thus, with a normally shaped ventricle and uniform contraction of the ventricular wall, the measurement obtained should accurately reflect left ventricular volume changes. The variability of this technique between the two investigators and the variability of successive measurements over the individual study period averaged 6%.

Repeated-measures analysis of variance was used to determine the significance associated with observed differences in the various dependent measures at the three time points (before pregnancy and 6 and 12 weeks post partum). If the overall analysis of variance resulted in statistical significance (p < 0.05), Fisher's least-significant difference procedure was used to further evaluate the specific comparisons of interest (before pregnancy vs 6 weeks post partum and before pregnancy vs 12 weeks post partum). Comparisons were performed on least-square means with a Bonferroni adjustment to control the type I error rate. Analyses were performed with SAS statistical software.

Results

The results (mean \pm SEM) of the serial cardiovascular measurements are presented in Table I. Enddiastolic volume was significantly increased at both 6 and 12 weeks post partum (16% and 12%). Similar significant increases were seen in stroke volume at both time periods (19% and 16%). Cardiac output was also elevated but to a lesser extent because of a 9% decrease in heart rate seen at 6 weeks post partum, but because the heart rate increased at 12 weeks post partum the increase in cardiac output (14%) was statistically significant at this time period. There was no difference in mean arterial pressure when compared at the different time periods. Systemic vascular resistance remained decreased at both 6 (-6%) and 12 (15%) weeks post partum with statistical significance attained at 12 weeks. The changes in maternal weight were variable. Although the group averaged an increase above pregravid weight of 6 kg at 6 weeks post partum and 3 kg at 12 weeks post partum, 30% and 38% were within 1 kg of pregravid weight at these study periods. There was no difference in cardiovascular response between those whose weight remained elevated over pregravid weight and those whose weight returned to pregravid levels. When the data were corrected for body surface area, the significance remained. There was no difference in cardiovascular response between primiparous and multiparous women.

Comment

Although there have been many serial studies of cardiovascular parameters during pregnancy, few have followed with serial measurements in the postpartum period. ^{1-3, 9, 10} This is the first report of these parameters measured by M-mode echocardiography that included the same population both before pregnancy and in the postpartum period. This study documents that some changes associated with pregnancy have not regressed by 12 weeks post partum when compared with values before pregnancy in lactating women. It is not known when these changes return to their baseline state or if there are permanent changes that occur as a result of

^{*}p < 0.01, compared with value before pregnancy.

 $[\]dagger p = 0.024$, compared with value before pregnancy.

pregnancy. There is accumulating evidence in both humans and animals that the cardiovascular system may undergo remodeling in response to pregnancy. Hart et al.11 have found that multiparous women have larger aortic diameters than do primiparous women, suggesting that there are alterations that occur during pregnancy and do not revert to their prepregnancy baseline. Robson et al.9 have documented differences in left ventricular wall thickness and mass between postpartum values and those obtained in a nonpregnant control group. Sady et al.10 found no difference in cardiac output and stroke volume when compared in the same women at 2 and 7 months post partum. Remodeling of the left ventricle has been shown to occur in the guinea pig during pregnancy12, 13 and during estrogen administration. 12 Davis et al. 14 demonstrated in the guinea pig that mean circulatory filling pressure does not change during pregnancy, but total body compliance and unstressed volume increases. These studies suggest that the pregnancy-induced increase in stroke volume and cardiac output may not be in response to increased preload by the Starling mechanism but that both the ventricle and the venous system have undergone remodelling. This concept has been supported by Hohman et al.¹⁵ in the rat model, who demonstrated structural changes in the vessel wall of the venous system in response to pregnancy. Thus permanent alterations in the cardiovascular system may be evoked by normal pregnancý.

Robson et al.9 have also studied hemodynamic changes serially from late pregnancy (38 weeks) through 24 weeks post partum (2, 6, 12, and 24 weeks). Initial significant decreases were found in all parameters studied by 2 weeks post partum but there was little change in stroke volume, end-diastolic dimension, and cardiac output between 6 and 12 weeks. This agrees with the trends seen in our group. They found no difference between breast-feeding and non-breastfeeding women. When compared with values in a nonpregnant control group, aortic and pulmonary valve area, aortic and mitral stroke volume, total left ventricular wall thickness, and left ventricular mass were significantly increased.

Indirect support for our findings of elevated stroke volume, end-diastolic volume, and possibly cardiac output in the postpartum period can be obtained by a comparison of the pregnancy study by Robson et al.5 with the postpartum study by the same investigator.9 The magnitude of the increases in stroke volume, enddiastolic volume, and cardiac output that were present when preconception values were compared with pregnancy were not matched by similar decreases when pregnancy values were compared with those found at 6 and 12 weeks post partum. Thus these parameters remain elevated above preconception values. These findings have important implications in the interpretation of previous studies1-3 of cardiovascular parameters during pregnancy. The postpartum period has been accepted as the baseline state; therefore the continued increase in stroke volume at this time period would serve to underestimate its contribution to the total increase in cardiac output seen in pregnancy.

M-mode echocardiography is a noninvasive technique that can serially follow changes in cardiovascular parameters.8. 16 Previous studies have documented excellent correlation of M-mode echocardiography with both angiography7, 16-derived stroke volume and thermodilution⁸ cardiac output. With a normally shaped ventricle and uniform contraction of the ventricular wall, the measurement obtained should accurately reflect left ventricular volume changes.9 In our experience with normal women, it has proved highly reproducible. In addition, the values obtained in our study for cardiac output and stroke volume are comparable to those of Robson et al.5 and Katz et al.2

The heart rate response seen in our study was variable. We observed a decrease (6 beats/min) at 6 weeks after delivery with an increase at 12 weeks to a level similar to preconception values. This disagrees with Robson et al.,9 who found no change throughout the puerperium yet agrees with Sady et al.,10 who in an exercising populace found an initial decrease in heart rate at 8 weeks compared with 7 months post partum.

In summary, we have found that left ventricular enddiastolic volume and stroke volume remain elevated over their values before conception when measured in the same populace both before and after a normal pregnancy. The effect of a pregnancy complicated by hypertension is an area for future study.

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Baroreflex function in normal pregnancy

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The sinoaortic baroreflex is one of the primary mechanisms that regulates blood pressure. Decreased baroreflex sensitivity has been reported in preeclampsia. We sought to determine whether pregnancy altered baroreflex sensitivity. From a radial artery catheter, heart rate and mean arterial pressure were recorded continuously onto a polygraph. The ratio of change in heart rate produced per unit of change in mean arterial pressure was calculated as an index of baroreflex sensitivity. Mean arterial pressure responses to incremental infusions of phenylephrine (0.4 to 2.0 μ g/kg/min) were measured in the same patients at term (n=9, 38.0 \pm 0.3 weeks) and again 6 to 8 weeks post partum (n=7). The results indicated (1) higher baroreflex sensitivity in pregnancy than in the postpartum period (0.9 vs 0.5 beats/min/mm Hg) (ρ < 0.007); (2) attenuated vascular responsiveness to α -adrenergic stimulation in pregnancy (ρ < 0.05); (3) a relationship between vascular responsiveness and baroreflex sensitivity. We conclude that pregnancy is associated with an increase in baroreflex sensitivity and that the attenuated response to phenylephrine is, at least in part, a result of increased baroreflex sensitivity. (AM J OBSTET GYNECOL 1991;165:886-90.)

Key words: Baroreflex sensitivity, baroreceptor, normal pregnancy

Short-term regulation of blood pressure is normally effected by the sinoaortic baroreflex. Stimulation of stretch receptors, which are located mainly in the aortic arch and carotid sinuses, causes reflex bradycardia and peripheral vasodilatation. The sensitivity of this reflex can be assessed from the ratio of change in

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heart rate produced per unit of change in blood pressure.^{1, 2}

Physiologic states such as sleep³ and exercise⁴ are associated with resetting of the baroreflex. Numerous studies⁵⁻⁷ also have reported baroreflex resetting with aging⁸ and after long-term exposure to chronic hypertension.

In addition to resetting of the barostat, certain conditions are associated with changes in reflex sensitivity. Loss of sensitivity implies weaker neuroanatomic control of the circulation in response to any acute perturbation.

Pregnancy-induced hypertension recently has been associated with loss of baroreflex sensitivity. 9. 10 The he-

modynamic instability characteristic of pregnancy-induced hypertension and the associated increased vascular reactivity to vasoactive agents may result from loss of baroreflex sensitivity.10

Seligman⁹ reported higher baroreflex sensitivity in normal pregnancy than in pregnancy-induced hypertension. However, the sensitivity during pregnancy was not compared with that of the nonpregnant state. We sought to quantify the baroreflex sensitivity in normal pregnancy and to determine whether pregnancy alters baroreflex sensitivity.

Material and methods

The protocol was approved by the Baylor College of Medicine Institutional Review Board for Human Investigation, and patients who participated in the study gave written consent. Nine carefully chosen patients with normal pregnancy at term were enrolled. Patients with medical problems or patients with any indication of an abnormal fetal heart rate pattern were excluded from this protocol. A reactive nonstress test and a biophysical profile score of 8/8 were required for entry into the study. The maternal age ranged from 17 to 31 years (mean \pm SD, 24 \pm 2), and the gestational age ranged from 36 to 39.4 weeks (mean \pm SD, 38.0 ± 0.3). Four of the nine patients were primigravid.

The radial artery was cannulated. Pressure transducers (Transpac II, Abbott Critical Care Systems, Sorenson, Chicago) were calibrated with the zero reference point at the midaxillary line. Continuous mean arterial pressure and heart rate were recorded on a twochannel recorder (model 2200S, Gould, Valley View, Ohio). Continuous fetal monitoring was performed during the entire study.

All measurements were recorded with the patient in a 15-degree left lateral decubitus position, and the head was raised to 30 degrees. For purposes of analysis, successive measurements of mean arterial pressure and corresponding heart rate were taken at baseline, every 10 minutes during the phenylephrine infusion, at the end of the infusion, and after a recovery interval for 30 minutes. Baroreflex sensitivity was derived from the change in heart rate associated with a change in mean arterial pressure during infusion of a pure α-adrenagonist, phenylephrine (Neo-Synephrine, Winthrop, New York).

Phenylephrine was diluted in 5% dextrose in water and infused at incremental doses ranging from 0.4 to 2.0 µg/kg/min. The rate of infusion was controlled with a variable rate Harvard infusion pump (Harvard, Dover, Mass.). The increment of phenylephrine was based on the individual patient's blood pressure response. Mean arterial pressure was not permitted to rise to >120 mm Hg. Data points for mean arterial pressure and heart rate were taken when the blood pressure had stabilized at a given dose. The same protocol was repeated 6 to 8 weeks post partum in seven of the nine patients.

Data are presented as mean \pm SD. Statistical analysis was performed with an analysis of variance (two-way) for baroreflex sensitivity and a Wilcoxon signed rank test for vascular reactivity. A paired t test was performed to compare the maximal decrease in heart rate during phenylephrine infusion, both before and after birth (n = 7). A p < 0.05 was taken as the level of significance.

Results

Antepartum initial mean arterial pressure and heart rate averaged 77.4 ± 2.0 mm Hg (range, 70.0 to 87.0) and 81.6 ± 3.6 beats/min (range, 66.0 to 93.0), respectively. Postpartum mean arterial pressure averaged 83.7 ± 2.6 mm Hg (range, 77.5 to 94.0) and postpartum heart rate averaged 65.0 ± 5.0 beats/min (range, 54.0 to 87.0). The individual subject's maximal decrease in heart rate during phenylephrine infusion was 28.1 ± 3.3 beats/min (range, 15.0 to 40.0 beats/min) before birth and 18.4 ± 4.7 beats/min (range, 3.0 to 39.0) after birth (paired t test, p < 0.02).

The changes in heart rate and mean arterial pressure associated with phenylephrine infusion are shown in Fig. 1. The comparison of the slopes of these regression lines defines the corresponding difference in baroreflex sensitivity during pregnancy and 6 to 8 weeks post partum. Pregnancy is associated with an increase in baroreflex sensitivity to 0.9 beat/min/mm Hg, compared with 0.5 beat/min/mm Hg at 6 to 8 weeks post partum (analysis of variance, p < 0.007). Reflex sensitivity was unrelated to baseline heart rate.

The use of phenylephrine infusion on the same patients before and after birth allowed us to establish a dose-response relationship between dose of phenylephrine and increase in mean arterial pressure. A considerable amount of interindividual variation was found in the slopes of change in mean arterial pressure (millimeters of mercury) per unit dose (micrograms per kilogram per minute) of phenylephrine. Pregnancy was associated with attenuated responses to α-adrenergic stimulation, with a change of 21.5 ± 3.0 mm Hg per unit dose of phenylephrine, compared with $31.3 \pm 6.6 \text{ mm}$ Hg per unit dose (micrograms per kilogram per minute) of phenylephrine post partum (p < 0.05, Fig. 2).

To determine whether the slopes of the individual phenylephrine dose-blood pressure response relationship were influenced by the baroreflex sensitivity, the

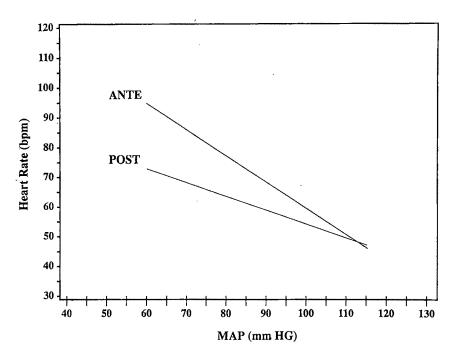


Fig. 1. Fitted curve of heart rate versus mean arterial pressure, including data from all patients. Slopes (ratio of change in heart rate produced per unit of change in mean arterial pressure) of two regression lines quantify baroreflex sensitivity (0.9 beat/min/mm Hg before birth [ANTE] vs 0.5 beat/min/mm Hg after birth [POST]; p < 0.007).

slopes of the change in mean arterial pressure (millimeters of mercury) per unit dose (micrograms per kilogram per minute) of phenylephrine were compared to ratio of change in heart rate produced per unit of change in mean arterial pressure. The relationship was defined by the following equation: y = -1.98x + 36.88; $r^2 = 0.33$, p = 0.0505. These data indicate that higher baroreflex sensitivity is associated with lower vascular responsiveness to α -adrenergic stimulation during pregnancy.

Comment

Many hemodynamic changes occur during pregnancy, including an increase in plasma volume and cardiac output that is accompanied by a fall in systemic vascular resistance and mean arterial pressure. Hence appropriate mechanisms to control the circulation and maintain the hemodynamic stability are required. The sinoaortic baroreflex represents the circulation's first defense against acute perturbations of systemic arterial pressure.

Seligman⁹ measured changes in pulse interval in response to elevation of blood pressure by phenylephrine or angiotensin and reported higher baroreflex sensitivity in normal pregnancy than in severe preeclampsia. However, he did not obtain measurements post partum. In our study the protocol was performed in the

same patient at term and again 6 to 8 weeks post partum. We found baroreflex sensitivity to be significantly higher in normal pregnancy at term than in the same subject post partum. This increased sensitivity may be of benefit during pregnancy in response to tilt and posture changes.

In studies with sheep, Magness and Rosenfeld¹¹ reported that at similar increases in mean arterial pressure the percent fall in cardiac output was greatest during pregnancy. The percent change in heart rate also was greatest in pregnant sheep during α -adrenergic receptor agonist stimulation.² These observations support the concept that normal pregnancy is associated with increased baroreflex sensitivity.

We assessed baroreflex sensitivity by means of the change in heart rate per unit change in mean arterial pressure. Higher baroreflex sensitivity was associated with a steeper slope, that is, a greater fall in heart rate per unit change in mean arterial pressure. Another characteristic of the sinoaortic baroreflex is the operating set point, that is, the blood pressure value that the baroreflex defends against any change. The lower baseline mean arterial pressure in pregnancy is perceived as normal. There is a shift away from the non-pregnant operating set point.

Our results indicated attenuated responses to α -adrenergic stimulation during pregnancy. These findings

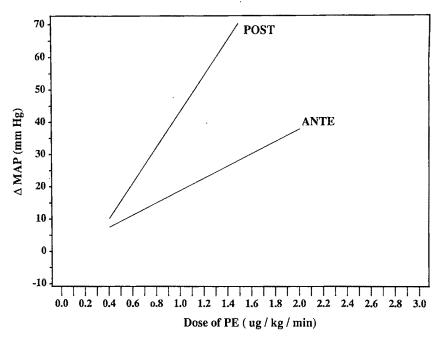


Fig. 2. Fitted curve of mean arterial pressure versus dose of phenylephrine infusion, including data from all patients. Slopes of change in mean arterial pressure (ΔMAP) (micrograms per kilogram per minute) of two regression lines quantify vascular responsiveness (21.5 ± 3.0 mm Hg per unit dose of phenylephrine before birth [ANTE] vs 31.3 ± 6.6 mm Hg per unit dose of phenylephrine after birth [POST] (p < 0.05).

are consistent with previous reports in animal models with infusions of norepinephrine, epinephrine, or phenylephrine.13-15 Similarly, the reduced pressor response to angiotensin II reported in pregnancy¹⁶ may be partly attributed to the increased baroreflex sensitivity. The phenomenon of reduced pressor responsiveness to angiotensin II in human pregnancy generally has been studied in relation to the dose of angiotensin II required to elicit a 20 mm Hg rise in blood pressure.16 Controversies exist about the exact mechanism by which attenuated pressor responses to angiotensin II appears to occur.17-19 It would be of interest to determine the extent to which baroreflex sensitivity correlates with the attenuated responsiveness to angiotensin in pregnant women.

Shepherd et al.20 explicitly demonstrated the relation between baroreflex sensitivity and vascular reactivity in the adult man. This association indicates the capacity of baroreflex function to buffer an increase in blood pressure. We also observed a relationship between these two parameters. However, we had a considerable amount of interindividual variation that could explain why our results did not reach a statistical significance. These data suggest that vascular reactivity is markedly attenuated in the presence of a sensitive sinoaortic baroreflex. Thus a patient with a lower baroreflex sensitivity would be more sensitive to vasoactive agents.

In summary, baroreflex sensitivity is increased in normal pregnancy at term, compared with that at 6 to 8 weeks post partum. The concomitant attenuated response to phenylephrine is probably in part related to this increased baroreflex sensitivity.

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Changes in immunologic parameters in normal pregnancy and spontaneous abortion

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To study the immunologic characteristics of miscarriage, 40 nonpregnant control subjects, 40 primigravid women in the first trimester of pregnancy, and 18 patients admitted with a spontaneous abortion were investigated. The total white blood cell count was raised significantly in normal pregnancy and spontaneous abortion (p < 0.0005). The lymphocyte count was unchanged. The total T-cell number fell significantly in normal pregnancy and abortion (p < 0.01). No change was seen in the cytotoxic suppressor or helper/inducer T-cell numbers. The number of activated T cells fell significantly in both groups of patients (p < 0.0005). The response to mitogens was greatly increased in both normal patients and those with miscarriage. A marked rise in interleukin-2 receptor levels was noted in patients with spontaneous abortion (p < 0.005). The changes in white blood cell count, total T-cell number, activated T-cell number, and mitogen activity were thought to be a direct result of pregnancy. The rise in interleukin-2 receptor levels was seen only in the miscarriage group. Although it is not known if these changes are cause or effect, it would appear that immunologic abnormalities are associated with spontaneous abortion. (Am J Obstet Gynecol. 1991;165:890-5.)

Key words: Normal pregnancy, spontaneous abortion, immunologic changes

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The fetus inherits genetic material from both parents; in spite of this, rejection usually does not occur. The mechanisms that prevent miscarriage are not fully understood. A change in the balance of maternal immunoregulatory cells toward greater suppression of the immune system could contribute to the immunoregulation of normal pregnancy. Approximately 15% of

pregnancies will end in miscarriage, usually in the first trimester of pregnancy. Once a fetal heart is seen on ultrasonographic examination, 95% to 98% of pregnancies will proceed successfully.1 Because fetal tissue is immunogenic, spontaneous abortion may have an immunologic cause.2 To investigate this possibility we have compared various immunologic parameters in three groups of women: nonpregnant women, normal pregnant women in the first trimester of pregnancy, and women admitted with noncontinuing pregnancy. The aim of this study was to assess whether any changes occur in T-cell subsets. B-cell function, mitogenic activity, and interleukin-2 (IL-2) receptor levels in pregnancy.

Methods

Forty nonpregnant, age-matched control subjects, 40 normal primigravid women in the first trimester of pregnancy, and 18 patients admitted with noncontinuing pregnancy were studied. The majority of the patients with noncontinuing pregnancy were recruited from the antenatal clinic after an ultrasonographic scan. In 12 women a diagnosis of blighted ovum was made; in five, the diagnosis was missed abortion; and one patient had an incomplete abortion. The parity ranged from 0 to 4, including two patients who had previously miscarried. The abortions all occurred in the first trimester of pregnancy. All patients gave informed consent before the investigation.

A total of 60 ml of blood was taken at the same time of day, and the following parameters measured: total white blood cell count (×109 cells/L), lymphocyte count (×10° cells/L), total T-cell number (×10° cells/L), helper/inducer T cells (×108 cells/L), cytotoxic/suppressor T cells (×10⁸ cells/L), activated T cells (×10⁸ cells/L), ratio of helper/inducer T cells to cytotoxic/suppressor T cells (×108 cells/L), mitogenic activity (tritiated thymidine), IL-2 receptors (U/ml), immunoglobulin G (ng/ml), and immunoglobulin M (ng/ml).

Peripheral blood lymphocytes. Heparinized blood was obtained from all control subjects and patients. The cells were then separated by density centrifugation with Ficoll-Hypaque³ and washed three times in minimal essential medium. The cells were then made to the required concentration in RPMI 1640 medium.

T-cell subset analysis. Samples were analyzed by a fluorescence-activated cell sorter (Becton Dickinson, Immunocytometry Systems, Mountain View, Calif.). This instrument is a flow cytometer that simultaneously measures electronic volume, wide-angle light scatter, and fluorescence signals from individual cells. The monoclonal antibodies used were supplied by Becton Dickinson. The CD3 monoclonal antibody reacts with total T cells; the CD4 monoclonal antibody, with helper/inducer T cells; the CD8 monoclonal antibody, with suppressor/cytotoxic T cells; and the Ia monoclonal antibody, with activated T cells. A differential white blood cell count was obtained for all patients, thus enabling the results to be expressed as absolute cell numbers.4

Response to mitogens. Peripheral blood lymphocytes were prepared in RPMI medium with 20% heatinactivated human serum (the same batch being used throughout) and made up to a concentration of either 1×10^6 cells per milliliter for phytohemagglutinin and concanavalin A or 2 × 106 cell per milliliter for pokeweed mitogen. All mitogens were used at optimal concentrations. Phytohemagglutinin, a helper T-cell mitogen, was used at a concentration of 10 µg/ml; concanavalin A, a suppressor T-cell mitogen, at a concentration of 100 µg/ml; and pokeweed mitogen, a B- and T-cell mitogen, at 0.2 μg/ml. All cultures were done in triplicate, and the cells were grown for 96 hours at 37° C, 95% humidity, and 5% carbon dioxide. Tritiated thymidine (1 µCi per well) was added for the last 4 hours of the incubation, and the cells harvested with a Dynatech cell harvester (Dynatech, Billinghurst, Sussex). The cells were then washed onto glass fiber disks with water and then fixed with 5% trichloroacetic acid and dried with methanol before the uptake of tritiated thymidine by peripheral blood lymphocytes.⁵ The interassay variability for the mitogenic assay was 7.2%. No positive control was used in the assay as the response to stimulation with pokeweed mitogen, phytohemagglutinin, or concanavalin A was sufficiently great, compared with that of controls (Fig. 1), that this was found not to be necessary.

IL-2 receptors. IL-2 receptors mediate the action of IL-2, an immune system growth hormone. Normal resting T and B cells do not display significant numbers of these receptors on their cell surfaces, but when these cells are stimulated by a challenge to the immune system, the number of IL-2 receptors on the plasma membranes of the cells increases and a form of the IL-2 receptor protein is released into the surrounding fluid by the activated cells. It is this soluble protein that is measured with a sandwich enzyme immunoassay kit (Laboratory Impex. Ltd., Teddington, Middlesex). The assay involves coating an anti-IL-2 receptor monoclonal antibody onto the wells of a microtiter plate. Soluble IL-2 receptor present in the sample or standard will then bind to the antibody on the wells. An enzymeconjugated anti-IL-2 receptor monoclonal antibody directed against a second epitope on the IL-2 receptor molecule is then added. This binds to the IL-2 receptor captured by the first antibody, thus completing the sandwich. Any unbound, enzyme-conjugated IL-2 receptor is removed by washing. A substrate solution is then added and the colored product formed is pro-

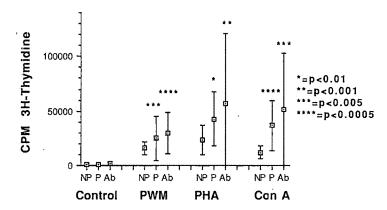


Fig. 1. Response of T cells in nonpregnant women (n = 40), normal pregnant women (n = 40), and patients with spontaneous abortion (n = 18) to control and three mitogens: pokewood mitogen (PWM), phytohemagglutinin (PHA), and concanavalin A $(Con\ A)$. Results expressed as counts per minute of tritiated thymidine uptake, showing $\pm 1\ SD$ by horizontal lines. Statistical significance is marked above each column. NP, Nonpregnant; P, normal pregnant; Ab, spontaneous abortion.

portional to the amount of IL-2 receptor present in the sample. This assay has an interassay variation of 3.7% and an intraassay variation of 11%.

B-cell studies. B-cell studies were performed as described previously. Briefly, peripheral blood lymphocytes were made to a concentration of 1×10^6 cells per milliliter in RPMI medium with 20% fetal calf serum (the same batch being used throughout the study). The amount of immunoglobulins G and M released from the cells into the culture medium was measured with an enzyme-linked immunosorbent assay.

Results are given as mean ± 1 SD. Statistical analyses were performed by means of a one-way analysis of variance.

Results

The results of the changes in T lymphocytes are shown in Table I. Although the total white blood cell count was significantly increased in normal pregnancy and in spontaneous abortion (p < 0.0005) compared with the nonpregnant controls, the lymphocyte count was unchanged. The total T-cell number was significantly reduced in the normal pregnant and abortion groups. There was no significant change in the numbers of helper/inducer or cytotoxic/suppressor T cells compared with those of nonpregnant women. The number of activated T cells was significantly lower in both groups (p < 0.0005). The ratio of helper/inducer T cells to cytototoxic/suppressor T cells was unchanged.

The changes in mitogenic activity are shown in Fig. 1. A significant rise in mitogenic activity to pokeweed mitogen, phytohemagglutinin, and concanavalin A was seen in both groups of patients when compared with nonpregnant control subjects. In the absence of mitogen there was no rise in T-cell activity.

There was no significant change in IL-2 receptor

levels in the normal pregnant group (Table II), but a significant rise (p < 0.001) was seen in the spontaneous abortion group.

The amount of immunoglobulin produced by mitogen-stimulated peripheral blood lymphocytes is shown in Table II. Immunoglobulin G and M production was significantly raised in the first trimester of normal pregnancy and in women with spontaneous abortion, although the latter was less significant because of the wide variation as shown by the large standard deviation.

Comment

For a pregnancy to continue the mother must tolerate fetal tissue that is foreign to her immune system. There are thought to be various protective mechanisms preventing immune attack.⁷

The two major cells of the immune system are T and B lymphocytes. While maturing in the thymic medulla, the T cells develop a receptor for antigens. Those destined to have cytotoxic/suppressor functions resemble the class I major histocompatibility complex antigens, whereas those with helper/inducer functions resemble class II major histocompatibility complex antigens. Foreign antigens will be recognized only if there is a major histocompatibility complex molecule on the cell surface that can be recognized by the T cells.⁸

A certain degree of genetic disparity between mother and fetus has been reported to benefit the developing pregnancy.⁹ A poorer outcome has been seen where there is excess antigen sharing in couples at the major histocompatibility complex, because this prevents recognition of the pregnancy as foreign and a helpful immune response is not produced.¹⁰ Sharing of minor histocompatibility or trophoblast-lymphocyte crossreactive antigen may be significant but is poorly understood.¹¹ Maternal recognition of incompatible

Table I. Results of changes in white blood cell count, total T-cell number, T-cell subsets, and ratio of helper/inducer T cells to cytotoxic/suppressor T cells in nonpregnant women (n = 40), normal pregnant women (n = 40), and patients admitted with spontaneous abortion (n = 18)

	White blood cell count	Lymphocyte count	T3	T4	T8	IA	T4:T8
Nonpregnant women Normal pregnant women Significance	6.4 ± 1.6 9.5 ± 1.9 p < 0.0005	2.2 ± 0.5 1.8 ± 0.4 NS	17.4 ± 4.1 14.1 ± 3.8 $p < 0.01$	· 10.2 ± 2.8 8.3 ± 2.6 NS	6.7 ± 1.6 6.0 ± 1.9 NS	3.1 ± 1.0 1.1 ± 1.1 $p < 0.0005$	1.52 ± 0.4 1.38 ± 0.5 NS
Women with spontaneous abortion Significance	7.9 ± 2.6 $p < 0.0005$	2.1 ± 0.6 NS	13.4 ± 4.8 $p < 0.01$	8.3 ± 3.2 NS	5.6 ± 2.6 NS	0.8 ± 0.7 $p < 0.0005$	1.48 ± 0.4 NS

T3, Total T cells; T4, helper/inducer T cells; T8, cytotoxic/suppressor T cells; IA, activated T cells; T4:T8, ratio of helper/inducer T cells to cytotoxic/suppressor T cells; NS, not significant.

Table II. Results of IL-2 receptor levels and immunoglobulin production in nonpregnant women, normal pregnant women, and patients admitted with spontaneous abortion

	IL-2 receptor	Immunoglobulin G	Immunoglobulin M
Nonpregnant women	415.8 ± 77.2 452.3 ± 126.4	159.5 ± 57.7 360.7 ± 313.8	233.9 ± 126.8 668.7 ± 583.6
Normal pregnant women Significance	452.5 ± 126.4 NS	p < 0.005	p < 0.005
Women with spontaneous abortion	685.0 ± 254.8	827.6 ± 1309.8	853.2 ± 1282.7
Significance	p < 0.005	p < 0.05	p < 0.05

NS, Not significant.

trophoblast-lymphocyte cross-reactive antigens on trophoblast could lead to the production of protective factors (e.g., blocking antibodies) beneficial to pregnancy.¹²

In our study the rise in total white blood cell count that was found in the pregnant women and patients with spontaneous abortion is a well-known effect of pregnancy, primarily caused by a neutrophilia.13

We found that alterations in the number of total and activated T cells were associated with both normal pregnancy and spontaneous abortion. This would appear to be an effect of pregnancy. Various changes in T-cell subset patterns have previously been reported. Sumiyoshi et al.14 found the number of helper/inducer T cells to be decreased and the number of cytotoxic/suppressor T cells to be increased in normal pregnancy. Threatened abortion was associated with a reduction in cytotoxic/suppressor T cells. In contrast, Vanderbeeken et al. 15 found a fall in the percentage of total Tcell numbers as a result of a decrease in helper/inducer T cells. The ratio of helper/inducer to cytotoxic/suppressor T cells also was decreased. Moore et al. 16 also found a decrease in helper/inducer T cells, although this was not statistically significant. The discrepancy between these earlier studies and this one may be due to expression of the results as percentages rather than absolute numbers. Alternatively, it may be related to the methods used or variations in patient populations.

We also found a fall in activated T-cell number, but

this was not the observation made by Vanderbeeken et al.,15 who found no change in the Ia antibody number in normal pregnancy.

To investigate the sensitivity of T cells, their response to pokeweed mitogen, phytohemagglutinin, and concanavalin A was studied. Like Sumiyoshi et al.,14 we found an increased response to mitogenic stimulation by lymphocytes of women with spontaneous abortion when compared with that of healthy pregnant women. However, unlike these authors we found the response in healthy women to be elevated when compared with that of control subjects, although it was significantly lower than the results found in spontaneous abortion. The reasons for this discrepancy are not clear at present. No increase was observed in the absence of mitogenic stimulation, It is possible that in normal pregnancy some cell stimulator is produced, and in spontaneous abortion this may be produced in excess.

One of the first events in the activation and release of T lymphocytes is the expression of IL-2 receptors on the surface that are subsequently released in a soluble form and the secretion of a lymphokine, IL-2, that exerts its biologic effects by interacting with the receptors.17 The receptor release is maximal around 6 days after T-cell challenge.18 It is known that IL-2 receptor levels may increase in renal graft rejection,19 and the prognosis of certain tumors, e.g., non-Hodgkin's lymphoma, may be monitored by IL-2 receptor levels.20 In this study no rise in IL-2 receptor levels was seen in the normal pregnant group; however, a significant rise was found in the group with spontaneous abortion. In a study performed by Stone et al.,21 three groups of women were studied after embryo or gamete transfer: those who failed to conceive, those who had a continuing pregnancy, and those who underwent spontaneous abortion. Blood was collected before transfer and on days 7 and 14 after embryo or gamete transfer, before full implantation would have occurred. At these times no significant differences in IL-2 receptor levels were found among the groups. In our study the changes were observed after the first missed menstrual period, during the first trimester of pregnancy, with ultrasonographic evidence of an implantation. The rise in IL-2 receptor levels indicates activation of the immune system and may reflect an immunologic rejection of the fetus as a cause of spontaneous abortion. It would have been interesting to see serial changes in the patients of Stone et al. who proceeded to miscarry and whether immunologic changes occurred at a later time.

It is not clear if the raised IL-2 receptor levels that we found in the patients with spontaneous abortion are causative or a result of a process that has become inevitable. However, monitoring of IL-2 receptor levels in patients with a threatened miscarriage may lead to a more accurate prediction of the outcome of the pregnancy.

Immunoglobulin production from peripheral blood lymphocytes was elevated in normal pregnancy and spontaneous abortion (Table II). Whether this is due to an increase in B-cell numbers or whether it is the result of increased production is not clear at present. There have been results in the literature of increased B-cell numbers occurring in normal pregnancy²²; however, these may have been inaccurate because the earlier tests interacted with monocytes, as well as with B cells.

These results suggest that, in addition to activation of T cells (as reflected by elevated IL-2 receptor levels), there is also activation of B cells in some cases of spontaneous abortion. At present it cannot be determined if these events occur singly or whether activation of one system leads to activation of the other. It would be unlikely that all cases of spontaneous abortion are caused by immunologic abnormalities. This is suggested by the wide range of results in our abortion group, with some results grossly abnormal and others within the normal range. Abnormality of the conceptus is another likely cause; however, chromosomal analysis of products of conception were not carried out in our study as it is only available for patients with a history of recurrent abortion. The possibility of altering the outcome of the pregnancy if an immunologic abnormality is identified may have practical implications.

Treatment of recurrent abortion with progesterone or human chorionic gonadotropin has previously been tried and various results have been noted.²³ Any treatment would have to commence as early as possible to allow the maximum benefit to the pregnancy.

Further studies looking at serial changes in normal pregnancy and changes in patients with a history of recurrent abortion being treated with human chorionic gonadotropin are currently under way in our unit. It is hoped that these will help to clarify some of our initial findings.

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High plasma cellular fibronectin levels correlate with biochemical and clinical features of preeclampsia but cannot be attributed to hypertension alone

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Current concepts of the pathogenesis of preeclampsia involve the generalized dysfunction of maternal vascular endothelial cells. We measured the endothelial isoform of fibronectin as a marker of endothelial cell injury throughout pregnancy in a prospective, case-control study. Nineteen women met strict criteria for the diagnosis of preeclampsia. Nineteen normal pregnant women, and 19 women with gestational hypertension but without other stigmata of preeclampsia (transient hypertension) were selected from the same cohort and matched according to race, age, nulliparity, and gestational age at delivery. Plasma levels of cellular fibronectin were significantly elevated in women meeting strict clinical and biochemical criteria for preeclampsia but not in women with normal pregnancies or transient hypertension. Moderate but significant elevations in mean levels were found in the second trimester in women destined to have preeclampsia, as compared with matched normal and transient hypertension groups ($\rho < 0.05$). The results indicate that elevated plasma levels of cellular fibronectin are not simply the result of increased blood pressure but reflect a maternal insult specific to the syndrome of preeclampsia. Elevation of the mean concentration during the midtrimester is consistent with the hypothesis that endothelial cell injury is a specific lesion that occurs early in the course of preeclampsia, before clinical signs and symptoms. (Am J Obstet Gynecol 1991;165:895-901.)

Key words: Endothelial cell injury, cellular fibronectin, preeclampsia

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Preeclampsia is a prevalent, pregnancy-specific disorder of unknown etiology and pathogenesis. It is defined as a syndrome manifested by gestational hypertension, excessive proteinuria, and generalized edema. Although increased blood pressure is normally the first manifestation noted clinically, recent studies suggest that hypertension per se is a less important pathogenetic feature of preeclampsia than are vasospasm and ischemia, which appear to mediate the significant maternal and perinatal morbidity associated with this syn-

drome. Moreover, transient hypertension, another hypertensive condition of pregnancy that is not associated with proteinuria, also does not carry the perinatal or maternal morbidity of preeclampsia. Unfortunately, it is often difficult to distinguish the early stages of these two conditions by clinical findings.

Because proteinuria, edema, activation of the coagulation cascade, and increased sensitivity to vasopressor agents may all be explained by endothelial dysfunction, we proposed that maternal vascular endothelial cell injury is a central and early feature in the development of the preeclamptic syndrome.24 This report describes our examination of cellular fibronectin, the endothelial cell-derived isoform of fibronectin, a plasma marker of endothelial cell injury, in the three trimesters of pregnancy. Fibronectins are a family of ubiquitous, highmolecular-weight extracellular matrix glycoproteins involved in a variety of fundamental tissue interactions, including cell adhesion and migration.5 These proteins are shed into the circulation, where they constitute a significant fraction of total plasma protein. The predominant isoform of circulating fibronectin is derived from hepatocytes.⁵ An isoform of fibronectin associated with endothelial cells and endothelial cell matrix in vivo, cellular fibronectin, is generated by alternative posttranscriptional processing of the fibronectin gene product. 5, 6 Cellular fibronectin is a minor component of circulating fibronectin, constituting <5% of the total plasma pool. The endothelium-derived protein bears two extra domains not found on the abundant hepatocyte-derived fibronectin.6 To assess the extent of maternal vascular endothelial cell injury during pregnancy, we have quantified circulating cellular fibronectin with a monoclonal antibody that selectively recognizes a conformational epitope near the extra domain-B (ED-B) region of endothelial-type cellular fibronectin.

We report that plasma cellular fibronectin concentrations are significantly elevated as early as the second trimester in women destined to have preeclampsia but not in women who have normal pregnancies or transient hypertension.

Material and methods

All pregnant women registering for prenatal care at Moffitt-Long Hospital of the University of California, San Francisco, and at San Francisco General Hospital since July 1988 have been invited to participate in a prospective study of preeclampsia. Blood samples were obtained serially throughout pregnancy at routine venipuncture. Such routine testing included the usual prenatal laboratory tests (first trimester), maternal α -fetoprotein screening (second trimester), glucose screening (late second or early third trimester), repeat hemoglobin and hematocrit screening (mid third trimester), and peripartum testing at labor and delivery

(late third trimester). Participating patients gave written informed consent for the sampling on a form approved by the University's Committee on Human Research. The blood samples were collected in sterile tubes containing ethylenediaminetetraacetate (EDTA) as an anticoagulant. The plasma was fractionated, and aliquots were coded and frozen at -70° C for later analysis. In samples collected at term, plasma specimens were obtained before intravenous hydration, magnesium sulfate infusion, or antihypertensive medication and thus were unaffected by differences in clinical management or therapy of parturients thought to have hypertensive conditions.

The women studied in this cohort were followed up throughout pregnancy, labor, delivery and for ≥12 weeks post partum with serial clinical observations, including measurements of blood pressure and proteinuria. Blood pressures were measured in the prenatal and postdelivery follow-up clinics with patients in an upright, seated position, with the first and fifth Korotkoff sounds. Intrapartum blood pressure results were obtained with the patient in the recumbent position with slight left lateral tilt and reflect the average of at least four measurements made before administration of medication or initiation of epidural anesthetic. At the completion of the final postpartum evaluation (≥12 weeks after delivery), the clinical and laboratory data were reviewed by a jury of clinical investigators who were blinded to the results of the plasma cellular fibronectin determinations. Each patient was assigned to one of three study groups: preeclampsia, normal, or transient hypertension. Patients with preeclampsia fulfilled criteria recommended by Chesley,7 namely, nulliparity, absence of a history of hypertension before pregnancy, increase in diastolic pressure of 15 mm Hg or systolic pressure of 30 mm Hg compared with blood pressures obtained before 20 gestational weeks, proteinuria ≥0.5 gm/24 hours or ≥30 mg/dl in a catheterized specimen, hyperuricemia >5.5 mg/dl (or 1 SD greater than the normal mean value before term8), and return to normal blood pressure and resolution of proteinuria by 12 weeks post partum. Patients with transient hypertension were defined by nulliparity, absence of hypertension before pregnancy, blood pressure criteria as defined above for preeclampsia but without proteinuria and hyperuricemia, and return to normal blood pressure within 12 weeks of delivery. Normal controls were selected from the same cohort of nulliparous parturients, in whom neither hypertension, proteinuria, nor hyperuricemia was observed throughout the study period. Criteria that resulted in the exclusion of patients from this study were the identification of any chronic metabolic disease, evidence of illicit drug use by urine toxicology screens, or the failure of elevated blood pressure, hyperuricemia, or proteinuria to

Table I. Demographic, clinical, and biochemical features of term pregnancies

	Preeclampsia (n = 19)	Normal (n = 19)	$Transient \\ hypertension \\ (n = 19)$
Maternal age at delivery (yr)	25 ± 2	29 ± 1	29 ± 2
Gestational age at delivery (wk)	39 ± 1	40 ± 1	39 ± 1
Serum creatinine (mg/dl)	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
Hematocrit (vol/vol, %)	36 ± 1	37 ± 1	37 ± 1
Placental weight (gm)	694 ± 26	680 ± 22	630 ± 37
Infant weight (gm)	3202 ± 121	3536 ± 105	$3124 \pm 175*$
Serum uric acid (mg/dl)	$7.0 \pm 0.3 \dagger$	3.9 ± 0.3	5.1 ± 0.6
Mean arterial pressure (mm Hg)	$108 \pm 3*$	77 ± 2	109 ± 4*
Platelet count (×1000/µl)	$223 \pm 20 \ddagger$	255 ± 19	277 ± 17
Plasma cellular fibronectin (µg/ml)	$9.5 \pm 1.2 \dagger$	3.5 ± 0.4	3.7 ± 0.7

These data (expressed as mean ± SE) were analyzed by analysis of variance with Fisher's post hoc comparisons between groups.

resolve within 12 weeks of delivery. Patients from each group were matched for race, age, and gestational age at delivery.

Plasma cellular fibronectin levels were determined by a specific enzyme-linked immunosorbent assay (ELISA) technique with the monoclonal antibody A134, which recognizes a conformational epitope near the ED-B region of cellular fibronectin (unpublished observations). Aliquots of each freshly thawed plasma sample were diluted 1:20 in sample buffer and assayed in duplicate with an intraassay coefficient of variation of 6%, an interassay coefficient of variation of 9%, and a sensitivity of $<0.1 \mu g/ml$. This assay was developed and performed at Adeza Biomedical. Plasma samples were collected prospectively throughout pregnancy as described above; however, first- and second-trimester specimens were not available for all patients. When possible, at least one sample from each trimester was obtained. When several samples from a single trimester were available for a given patient, an average level for that trimester was calculated (<13 menstrual weeks, first trimester; 13 to 26 weeks, second trimester, ≥27 weeks, third trimester). The mean (±SE) plasma cellular fibronectin concentration in 50 consecutive specimens obtained from an independent group of normal pregnant women was $3.8 \pm 0.1 \,\mu\text{g/ml}$ in the same laboratory.

Confirmation of the specificity of the A134 antibody was afforded with a combination of isoelectric focusing, membrane transfer, and ligand blotting. Briefly, protein G-purified A134 monoclonal antibodies were electrofocused at 8 mA constant current in polyacrylamide gels containing a mixture of ampholines (pH 3 to 10 and pH 5 to 7, 1:4, vol/vol), 1% NP-40, and 10% sorbitol. The proteins were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, N.H.) and

replicate blots were incubated with 10 μg/ml of either cellular fibronectin isolated from human amniotic fluid9 or plasma fibronectin (Boehringer-Mannheim, Indianapolis). A polyclonal rabbit antihuman total fibronectin antiserum (A120, Adeza Biomedical, which recognizes a common determinant in all forms of human fibronectin) conjugated to alkaline phosphatase was subsequently incubated with the membrane filters in the presence of substrate (5-bromo-4-chloro-3-indolyl phosphate, Sigma Chemical Co., St. Louis) to identify cellular fibronectin-specific bands on the immunoblots.

Experiments to document the expression of cellular fibronectin by human endothelial cells were performed with primary human umbilical vein endothelial cell cultures as previously described.2 In some experiments matched cultures of human umbilical vein endothelial cells were exposed to bacterial endotoxin (lipopolysaccharide from Escherichia coli serotype 055:B5, 1 µg/ml, Sigma) for 24 hours, to provide a model of endothelial cell activation or injury. The human umbilical vein endothelial cells were washed with phosphate-buffered saline solution (pH 7.4) and lysed in a hypotonic solution of 10 mmol/L Tris, 1 mmol/L EDTA (pH 7.4) with repeated tituration through a 22-gauge syringe. The cell lysates were assayed for cellular fibronectin content by ELISA and total cellular protein was determined by the Bradford method.11

Statistical tests performed in these studies included descriptive, multiple comparison and correlation analyses of clinical and biochemical parameters among preeclampsia, normal, and transient hypertension groups. Results of this study are reported as the group mean \pm SE. Analysis of variance with Fisher's multiple comparison post hoc tests were used to assess differences among the three groups of patients. In some cases

^{*}Value differs significantly from normal group (p < 0.05).

[†]Value differs significantly from normal and transient hypertension groups (p < 0.05).

[‡] Value differs significantly from transient hypertension group (p < 0.05.

Table II. Longitudinal assessment of plasma cellular fibronectin concentrations, blood pressure, and hemoglobin during pregnancy

	First trimester $(n = 16)$	Second trimester $(n = 25)$	Third trimester $(n = 57)$
Plasma cellular fibronectin (µg/ml)			-
Preeclampsia	1.6 ± 0.6	$2.7 \pm 0.4*$	$8.7 \pm 1.1*$
Normal	1.9 ± 0.4	1.8 ± 0.2	3.3 ± 0.4
Transient hypertension	1.6 ± 0.4	1.2 ± 0.2	3.5 ± 0.7
Mean arterial pressure (mm Hg)			
Preeclampsia	81 ± 3	82 ± 2	$94 \pm 3 \dagger$
Normal	80 ± 3	81 ± 1	83 ± 1
Transient hypertension	86 ± 2	84 ± 1	$95 \pm 2 †$
Hemoglobin (gm/dl)			
Preeclampsia	ND	11.9 ± 0.3	12.2 ± 0.3
Normal	ND	12.0 ± 0.4	12.8 ± 0.3
Transient hypertension	ND	12.1 ± 0.3	12.7 ± 0.4

These data (expressed as mean \pm SE) were analyzed within each trimester by analysis of variance with Fisher's post hoc comparisons between groups. ND, Not determined.

simple correlation analyses were performed. When longitudinal differences throughout pregnancy were assessed, clinical and biochemical data for each patient were averaged to yield a single value for each trimester of pregnancy. Prepartum cellular fibronectin concentrations were analyzed with diagnostic group and the presence of labor as main effects in a two-way analysis of variance to examine the possible interaction of labor on cellular fibronectin levels. Tests with p < 0.05 were accepted as significantly different.

Results

Nineteen women met the stringent requirements for the diagnosis of preeclampsia. From the same cohort, 19 normal controls and 19 women with transient hypertension were selected and matched to the preeclamptic patients. The results in Table I show the mean ± SE values of the clinical and biochemical findings in the late third trimester, immediately before delivery. As expected from the criteria used to define the three groups, blood pressures were significantly elevated in the preeclampsia and transient hypertension groups relative to normal controls. Similarly, the mean proteinuria and uric acid levels were greater in preeclamptic pregnancies than in either of the remaining groups (p < 0.05). By contrast, maternal age, gestational age, hematocrit, creatinine concentration, and placental weights were not different among the three groups in this patient population. The mean platelet count in the preeclampsia group was slightly, but significantly, less than that of the transient hypertension group. The mean infant weight in transient hypertension pregnancies was statistically less than that in normal pregnancies (Table I). The interval between initial presumptive diagnosis and onset of labor was $3.4\,\pm\,1.2$ days and 10.4 ± 2.9 days for the preeclampsia and

transient hypertension groups, respectively. The preeclampsia group manifested diagnostic hypertension 6.9 ± 1.7 days before the onset of labor. As discussed previously, however, assignment of the final diagnoses was made after 12 weeks post partum.

The results of the plasma cellular fibronectin analyses, shown in Fig. 1, demonstrated that mean cellular fibronectin levels were significantly greater in preeclampsia (9.5 \pm 1.2 μ g/ml) than in matched normal $(3.5 \pm 0.4 \,\mu\text{g/ml})$ or transient hypertension $(3.7 \pm 0.7 \,\mu\text{g/ml})$ $\mu g/ml$) pregnancies at term (n = 57, p < 0.05). Of these patients, 61% (23/57) were in labor at the time of the final prepartum blood sample. Neither the presence nor absence of labor nor the interaction between the study groups and labor significantly affected the cellular fibronectin concentration (F = 0.87, p > 0.42, two-way analysis of variance, n = 57). When cellular fibronectin concentrations were analyzed independently of the patient groups, they were found to correlate positively with concentrations of urine protein, serum uric acid and creatinine (r > 0.56, p < 0.001) and negatively with platelet count and infant weight (r > 0.28, p < 0.05).

The mean plasma cellular fibronectin levels of each patient group were compared during each of the three trimesters of pregnancy (Fig. 2). While we found no statistical differences among patients in the first trimester, average levels were elevated by the second trimester in women destined to have preeclampsia, relative to matched normal and transient hypertension groups (p < 0.05). By contrast, the traditional clinical parameter of blood pressure achieved diagnostically significant levels only during the third trimester (Table II). The hemoglobin concentrations in these patients were indistinguishable among the three groups in both the second and third trimesters (Table II). In the imme-

^{*}Value differs significantly from normal and transient hypertension groups (p < 0.05).

[†]Value differs significantly from normal group (p < 0.05).

diate postpartum period (≤48 hours after delivery) cellular fibronectin levels tended to decrease but remained statistically higher in the preeclamptic group (6.2 \pm $0.4, 3.6 \pm 0.4, \text{ and } 3.2 \pm 0.7 \,\mu\text{g/ml}, n = 56, \text{ for pre-}$ eclampsia, normal, and transient hypertension groups, respectively).

The specificity of A134 was demonstrated by isoelectric focusing of the monoclonal antibodies and transferring these to nitrocellulose filters. With the A134 immunoglobulin fixed to nitrocellulose, the monoclonal antibody binds a cellular isoform of fibronectin present in amniotic fluid but not in plasma fibronectin (Fig. 3). Identical data were obtained with these and other purified preparations of cellular fibronectin and plasma fibronectin when A134 immunoglobulin was fixed to microtiter plates with the enzyme-linked immunosorbent assay format (data not shown).

To ascertain that the cellular fibronectin epitope recognized by the monoclonal antibody A134 was indeed expressed by human endothelium, cultures of normal human umbilical vein endothelial cells were grown to confluence in media reconstituted with 20% fetal calf serum, washed, lysed, and assayed for cellular fibronectin production by enzyme-linked immunosorbent assay. Neither fetal calf serum containing media nor lysis buffer had detectable cellular fibronectin. Human umbilical vein endothelial cells cultured under normal conditions contained 0.46 µg cellular fibronectin per milligram cellular protein. In identical cells exposed to 1 μg/ml bacterial endotoxin in vitro the concentration increased to 0.90 µg cellular fibronectin per milligram cellular protein.

Comment

Several clinical and biochemical findings support the hypothesis that maternal vascular endothelial cell injury plays an important role in the pathogenesis of the preeclampsia syndrome. The increased rate of disappearance of circulating Evans blue dye, 12 elevations of factor VIII antigen,13 decreased levels of prostacyclin,14 and the pathognomonic renal biopsy findings15 in these women suggest vascular endothelial cell dysfunction. Studies from our own laboratory have provided both direct² and indirect³ evidence of endothelial cell injury in this condition. Previous studies, using total¹⁶ and cellular17 plasma fibronectin as markers, demonstrated significant differences between normal and preeclamptic pregnancies.

This investigation was designed to compare the plasma concentrations of cellular fibronectin among normal pregnant women, patients with unambiguous preeclampsia, and pregnant women with transient hypertension as defined under the American College of Obstetricians and Gynecologists' classification of hypertensive disorders in pregnancy18 and in a recent re-

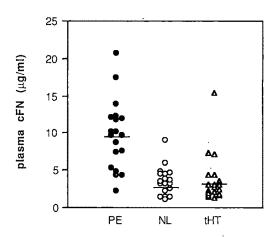


Fig. 1. Distribution of plasma cellular fibronectin (cFN) levels in preeclampsia (PE, •), normal (NL, o), and transient hypertension (tHT, Δ) pregnancy groups was determined at term. Nineteen patients with preeclampsia and 19 patients each in the normal and transient hypertension groups were studied (some overlapping values are not resolved in scattergram). Horizontal lines indicate mean cellular fibronectin concentration of each group.

vision of these criteria.19 The latter diagnosis, which currently can be made only retrospectively, tends to recur in subsequent pregnancies and is a harbinger of eventual essential hypertension. Previous studies suggest that it is not associated with increased maternal or fetal morbidity.1 In our study the mean interval between the first observation of diagnostically elevated blood pressure and the onset of labor was similar in preeclampsia (6.9 days) and transient hypertension (10.4 days) groups (p > 0.22), although on average complete diagnostic criteria for preeclampsia were not met until 3.4 days before labor. This observation reflects the aggressive management of preeclamptic pregnancies in our institutions once the diagnosis has been established. Additionally, however, the longer interval in transient hypertensive pregnancies indicates that these women differ remarkably from their normal peers, with substantial blood pressure elevation before the onset of uterine contractions.

In the second and third trimesters of pregnancy, cellular fibronectin levels were significantly greater in patients ultimately classified as having preeclampsia, relative to matched normal and transient hypertension pregnancies in the same cohort. That this finding was not noted in pregnancies complicated by transient hypertension indicates that elevated blood pressure per se is not responsible for the increase in plasma cellular fibronectin levels of preeclampsia. Significant elevations were detectable as early as the second trimester in women destined to have preeclampsia, suggesting that maternal vascular endothelial cell injury, as detected by the release of an endothelial isoform of fi-

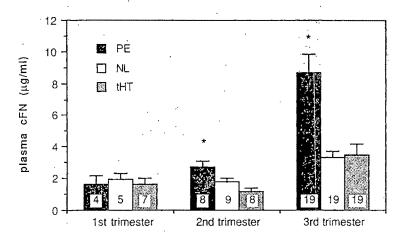


Fig. 2. Prospective analysis of plasma cellular fibronectin levels was performed throughout pregnancy. Average concentrations were determined for each trimester in the same preeclampsia (PE, dark histograms), normal (NL, open histograms), and transient hypertension (tHT, stippled histograms) groups. Cellular fibronectin levels in plasma from women destined to have preeclampsia were statistically greater in second- and third-trimester samples relative to the other two groups (p < 0.05, analysis of variance with Fisher's test). Values at base of each histogram indicate number of patients assessed at that gestational interval.

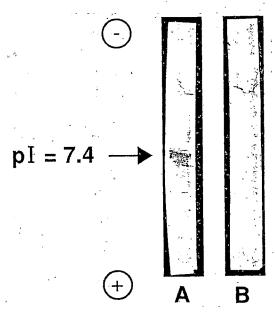


Fig. 3. Electrofocusing—Western ligand blot of A134 monoclonal antibody. Affinity-purified A134 monoclonal antibodies underwent electrofocusing and were transferred to nitrocellulose as described in Material and methods. Filters were incubated with either amniotic fluid fibronectin (lane A) or plasma fibronectin (lane B), and polyclonal antifibronectin antiserum (A120, conjugated to alkaline phosphatase) was added. Deposition of a colored reaction product demonstrated that A134, which was focused at isoelectric point = 7.4, recognizes a cellular isoform of fibronectin in amniotic fluid (lane A), but the antibody failed to bind plasma fibronectin (lane B).

bronectin, is an early and specific phenomenon in the pathogenesis of preeclampsia.^{2,20} Thus one of the most important findings of this study is that a fundamental difference exists between the pathophysiologic char-

acteristics of transient hypertension and that of preeclampsia. Chesley,⁷ among others, has long admonished against including patients with nonproteinuric hypertension in clinical investigations of preeclampsia. This precaution may be particularly relevant to studies from some European and British centers that, according to the diagnostic criteria proposed by Nelson,²¹ do not require proteinuria for the diagnosis of preeclampsia.

As our cellular fibronectin data are presented as circulating concentrations (micrograms per milliliter), a possible explanation for the apparent increase in pre-eclamptic women could be secondary to the reduced plasma volume reported in this condition. In this study no statistical differences in hemoglobin concentrations or hematocrit levels among the three groups were detected, indicating that cellular fibronectin levels expressed as a specific activity (e.g., micrograms cellular fibronectin per gram hemoglobin) also are elevated significantly in the second and third trimesters in women destined to have preeclampsia. Thus hemoconcentration per se cannot explain the observed increase in cellular fibronectin concentration.

An unanswered question is whether cellular fibronectin quantification may provide a marker as a midtrimester screening test for preeclampsia. To date, the rollover test,²² response to angiotensin II infusion,²³ and factor VIII antigen concentrations¹³ have been suggested as predictive tests, yet none has seen widespread utility. Lazarchick et al.¹⁶ reported that elevations in total fibronectin may be predictive in women destined to have preeclampsia. As plasma total fibronectin primarily reflects release of hepatic fibronectin, measurement of the endothelial cell isoform might provide an earlier and more specific marker of preeclampsia, as

demonstrated recently by Lockwood and Peters. 17 However, the overlap of second-trimester values in these preliminary studies suggests that cellular fibronectin is unlikely to be clinically useful as a cross-sectional predictor of preeclampsia.

We are in the process of extending this prospective, blinded analysis to ascertain whether longitudinal assessment of cellular fibronectin in individual patients can predict the 5% to 10% of pregnant women expected to have preeclampsia.1 Previous trials of high-risk patients suggest that tests with positive predictive values of 20% to 40% would identify appropriate candidates for prophylactic, low-dose aspirin therapy.24 Moreover, with increasing evidence that progressive maternal endothelial cell dysfunction is responsible for many of the pathophysiologic findings of the preeclamptic syndrome, we are searching for the circulating factor(s) that incites this process in vivo. Once such a factor(s) is identified, rational and specific therapy designed to reverse the pathologic processes involved in preeclampsia should be forthcoming.

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The effect of maternal hemodynamics on fetal growth in hypertensive pregnancies

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Seventy-six pregnancies in which hypertension complicated pregnancy before 28 weeks' gestation were studied. In 36, hemodynamics were characterized by increased cardiac output and low vascular resistance; in 32, hemodynamics were characterized by high resistance; in eight hemodynamics crossed over from high output to high resistance during pregnancy. High-resistance hypertension was associated with a mean birth weight 1058 gm less than that in the low-resistance group (p=0.001). The reduction in birth weight was due to a 4-week difference in gestational age (p=0.001) and lower percentile weights for gestational age, 19th versus 39th (p=0.005). Infants in the crossover group had low percentile weights and a high rate of intrauterine fetal death. (AM J OBSTET GYNECOL 1991;165:902-6.)

Key words: Hypertension, intrauterine growth retardation, cardiac output

Maternal hypertension is associated with increased risk of compromised fetal growth and intrauterine death. However, adverse fetal effects are not uniform; some pregnancies complicated by hypertension severe enough to require delivery for maternal indications produce infants that are normally grown without evidence of uteroplacental insufficiency. Over the past 6 years we have routinely measured the cardiac output noninvasively in most hypertensive pregnancies. Clinical experience has led us to hypothesize that the incidence of impaired fetal growth in hypertensive pregnancies is related to the individual character of maternal hemodynamics. Specifically, high-resistance hypertension seems to be associated with an increased incidence of intrauterine growth retardation while high cardiac output, low-resistance hypertension is frequently associated with normal fetal growth. The following retrospective study was designed to test this hypothesis.

Material and methods

We reviewed our hemodynamic data to identify pregnancies where hypertension was diagnosed at ≤28 weeks of gestation. Hypertension was defined as a diastolic pressure of 90 mm Hg on two occasions 6 hours apart. Screening blood pressures were measured by

auscultation and the fifth Korotkov sound. We chose to study women whose hypertension was evident early in pregnancy so that potential fetal effects would be maximized. We hypothesized that fetal effects would be related to the pattern of maternal hemodynamics; therefore we did not analyze the data on the basis of the presence or absence of chronic hypertension or proteinuria. Given the high rate of underlying maternal disease associated with early hypertension in pregnancy, we thought that these characterizations would frequently be inaccurate.

Cardiac output was measured by Doppler technique with the woman in a left lateral semirecumbent position. We have previously validated the accuracy and reproducibility of this technique in pregnancy.^{2,3} Blood pressure was measured by automated cuff. Mean arterial pressure and total peripheral resistance were calculated.

Pregnancies were categorized on the basis of the first hemodynamic measurement made after the end of the first trimester. High-resistance hypertension was defined by a total peripheral resistance ≥ 1150 dynes · cm · sec⁻⁵. Low resistance hypertension was defined by a total peripheral resistance <1150 dynes · cm · sec⁻⁵.

The hemodynamics of patients on antihypertensive therapy were examined to determine if therapy would be likely to result in an inappropriate categorization. For example, a patient with high cardiac output, low-resistance hypertension treated with a β -adrenergic receptor blocker could be accurately placed in the low-resistance group; lowered resistance would not be expected to be the result of β -adrenergic receptor blockade. On the other hand, a patient with low-resistance hypertension treated with a vasodilator such as hy-

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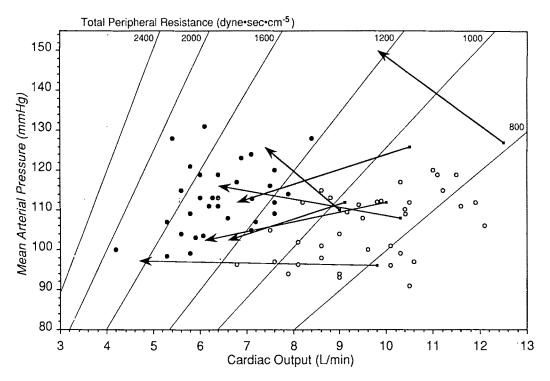


Fig. 1. Maternal hemodynamics. Mean arterial pressure of each subject is plotted against cardiac output. Isometric lines of vascular resistance are included so that all three parameters can be displayed on a single figure. Closed circles, High-resistance subjects; open circles, low-resistance subjects; closed squares, subjects whose hemodynamics crossed over. Associated vector graphically displays change in hemodynamics associated with crossover.

Table I. Maternal characteristics

	Low-resistance (n = 36)	p _i	High resistance (n = 32)	þ ₂	Crossover (n = 8)	<i>þ</i> 3
Age (yr)	29.3 ± 5.7	NS	28.5 ± 5.5	NS	29 ± 6.2	NS
Weight (kg)	94.3 ± 25.3	0.001	75.5 ± 17.6	NS	87.6 ± 5.2	NS
Nulliparous	19 (53%)	NS	13 (41%)	NS	1 (13%)	NS
Chronic hypertension	20 (56%)	NS	21 (66%)	NS -	6 (75%)	NS
Proteinuria	19 (53%)	NS	15 (47%)	NS	3 (38%)	NS

Values are mean \pm SD. p_1 , Low-resistance versus high-resistance; p_2 , high-resistance versus crossover; p_3 , crossover versus lowresistance; NS, p < 0.05.

dralazine could not be accurately characterized. These patients were excluded from the study.

All patients had follow-up with hemodynamic measurements until the end of pregnancy. In a small number of cases we observed that in subsequent measurements the hemodynamic pattern crossed over from low-resistance to high-resistance hypertension. These pregnancies were placed in a third group, crossover.

Birth weights were abstracted from the mothers' medical charts. Gestational age was determined by best obstetric parameter. Percentile birth weight was calculated from normative data for births at sea level.4 Intrauterine growth retardation was defined as birth weight at <10th percentile for gestational age.

Statistical analysis was performed by analysis of variance, χ^2 , and Fisher's exact tests.

Results

Seventy-six pregnancies that met study criteria were identified: 36 low-resistance, 32 high-resistance, and eight crossover. Only one could not be adequately characterized because of hypertensive therapy. Maternal characteristics are listed in Table I. Women with lowresistance hypertension were 19 kg heavier than those with high-resistance hypertension (p < 0.001). Women who crossed over were intermediate in weight between the two groups. The rates of nulliparity, chronic hypertension, and proteinuria were similar among the groups.

Initial individual maternal hemodynamics are displayed graphically in Fig. 1, where mean arterial pressure is plotted against cardiac output. Summary statistics are listed in Table II. The groups were partitioned

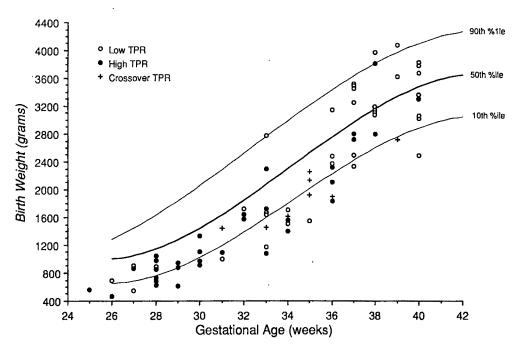


Fig. 2. Fetal growth. Individual birth weights are plotted against gestational age. The 10th, 50th, and 90th percentiles of normal birth weights at sea level are included for reference. *Closed circles*, Infants from high-resistance pregnancies; open circles*, infants from low-resistance pregnancies; plus signs, infants from crossover pregnancies.

Table II. Maternal hemodynamics

	Low-resistance	p ₁	High-resistance	<i>p</i> ₂	Crossover	p ₃
Heart rate (beats/min)	93 ± 14	0.001	79 ± 14	0.05	91 ± 14	NS
Mean arterial pressure (mm Hg)	106 ± 8.4	0.001	113 ± 8.6	NS	115 ± 15	0.01
Cardiac output (L/min)	9.6 ± 1.3	0.001	$6.4 \pm .91$	0.01	9.8 ± 1.5	NS
Stroke volume (ml/min)	105 ± 17	0.001	84 ± 16	0.01	105 ± 15	NS
Total peripheral resistance (dyne · cm · sec ⁻⁵)	862 ± 166	0.001	1427 ± 199	0.001	974 ± 111	NS

Values are mean \pm SD. p_t , Low-resistance versus high-resistance; p_2 , high-resistance versus crossover; p_3 , crossover versus low-resistance; NS, p > 0.05.

by vascular resistance, so the differences in resistance, cardiac output, and stroke volume were expected. The mean arterial pressures of both the high-resistance and crossover groups were higher than that of the low-resistance group (p < 0.001, p < 0.01). Mean heart rates of the low-resistance and crossover groups were higher than that of the high-resistance group (p < 0.001, p < 0.05).

Birth weight is plotted against gestational age for each study infant in Fig. 2. Summary statistics are listed in Table III. Infants of mothers with high-resistance hypertension were delivered 4 weeks earlier than those of women with low-resistance hypertension (p < 0.001). Given the large difference in gestational age, a large difference in birth weight was expected (p < 0.001). The mean percentile birth weight of infants born to women with high-resistance hypertension was 19th compared with 39th for infants born to women in the low-resistance group (p < 0.005). Differences in

the rates of intrauterine growth retardation corresponded to differences in percentile birth weights.

The mean gestational age of infants in the crossover group was similar to that in the low-resistance group. However, the infants in the crossover group exhibited growth retardation similar to that seen in the high-resistance group. In addition, they experienced a very high rate of fetal death (38%). In one case antepartum testing was very abnormal, but the patient repeatedly refused admission to the hospital. In the other two the patients had reactive nonstress tests without decelerations <3 days from the fetal death.

Comment

The hemodynamics of hypertension in pregnancy have been the subject of considerable controversy. Previous notions that vasoconstriction uniformly mediates all hypertensive disease in pregnancy have been challenged by numerous cross-sectional studies of patients

Table III. Neonatal outcome

	Low-resistance	p_1	High-resistance	p ₂	Crossover	p ₃
Gestational age (wk)	35.7 ± 4.0	0.001	31.7 ± 4.0	0.05	34.8 ± 2.3	NS
Weight (gm)	2548 ± 1047	0.001	1490 ± 868	NS	1921 ± 440	NS
Intrauterine growth retardation	8 (22%)	0.03	14 (44%)	NS	5 (63%)	0.02
Weight (%)	39 ± 32	0.005	19 ± 20	NS	14 ± 10	0.05
Fetal death	0 (0%)	NS	2 (6.3%)	0.05	3 (38%)	0.005
Neonatal death	3 (8.3%)	NS	2 (6.3%)	NS	0 (0%)	NS

Values are mean \pm SD. p_1 , Low-resistance versus high-resistance; p_2 , high-resistance versus crossover; p_3 , crossover versus lowresistance; NS, p > 0.05.

near delivery5-11 and by our recent longitudinal study of uncomplicated nulliparous patients who developed preeclampsia.12 In this study we have documented the marked heterogeneity of hemodynamics exhibited by women who become hypertensive early in pregnancy (Fig. 2). We have also documented the potential for women with high-output, low-resistance hypertension to cross over to high-resistance hypertension in the course of pregnancy. We have previously suggested that this Guytonean model of hypertension,18 where an individual may progress from high-output to high-resistance hypertension, may explain the complexity and heterogeneity of hypertension in pregnancy. 12, 14

We examined the relationship between fetal outcome and the hemodynamic characteristics of the mothers' hypertension. Infants born to women with high-resistance hypertension required delivery for fetal or maternal indications 4 weeks earlier in pregnancy. In addition, these infants were much smaller for gestational age. Increased prematurity, coupled with an increased rate of growth retardation, resulted in infants with dramatically smaller birth weights. These findings are consistent with those of Nisell et al.15 We hypothesize that global vasoconstriction in women with high-resistance hypertension is associated with decreased uteroplacental blood flow and poor fetal growth.

In Fig. 2 one can see that impaired fetal growth is nearly uniform in the high-resistance group. Only two infants were above the 50th percentile. However, such uniformity is not present in the low-resistance group. Although many infants were normally grown and a few were macrosomic, eight (22%) were at <10th percentile. Despite a global condition characterized by increased perfusion, the uteroplacental bed in these pregnancies seems to be underperfused; aberrations in the regional circulation of some women in the low-resistance group may not be adequately characterized by assessment of total peripheral resistance. We hypothesize that the vessels of the uteroplacental bed are more susceptible to hemodynamic injury than the remainder of the circulation and therefore may cross over to a high-resistance state earlier than the systemic circulation as a whole.

The group of patients whose systemic hemodynamics

crossed over are particularly interesting. However, the small size of the group has limited statistical power, and the results should therefore be interpreted cautiously. Initially, the patients in the crossover group were similar to patients in the low-resistance group. Their mean cardiac output and mean heart rate were the same. They did have a higher mean arterial pressure, suggesting more severe hypertension.

Infants from this group were different from each of the other groups. First, they were undergrown, comparable to the high-resistance group. The degree of growth retardation suggests that alterations in uteroplacental blood flow had been present for a considerable period of time while the systemic circulation was hyperdynamic. Second, shortly after systemic hemodynamic crossover we observed a high rate of intrauterine fetal death. In two cases the observable change in fetal environment was very abrupt and corresponded to systemic vasoconstriction. In these patients, before vasoconstriction, the underlying degree of uteroplacental insufficiency was adequately compensated. In this context an abrupt reduction in blood flow associated with systemic vasoconstriction had lethal consequences. We were impressed with the rapidity of deterioration of the fetal environment. At present in our practice the crossover of maternal hemodynamics in hypertensive patients is considered a critical event that requires intense fetal surveillance.

Many of the patients in the study, if managed expectantly, were treated with antihypertensive agents. Could antihypertensive therapy be responsible for some of the findings in this study? We believe that this is unlikely. In general, patients in the high-resistance group were treated with vasodilators, primarily hydralazine. Patients in the low-resistance group were treated with a β-adrenergic receptor blocker, primarily atenolol. If the patients' hemodynamics changed during the course of therapy, the antihypertensive regimen was altered accordingly. We have previously demonstrated that hydralazine and atenolol, when used in high- and low-resistance groups, respectively, have predictable effects on maternal hemodynamics.16 Hydralazine lowers resistance and atenolol tends to increase resistance; therefore the effects of these drugs would work to reduce differences between groups rather than to increase them.

We have demonstrated clear correlations between fetal outcome and maternal hemodynamics in hypertensive patients. A vasoconstricted maternal circulation is associated with early delivery and impaired fetal growth. In addition, we present evidence to suggest that the uteroplacental circulation may adjust independently from the maternal circulation; being more sensitive to forces that lead to vasoconstriction, regional crossover may occur earlier in pregnancy. Crossing over to high-resistance hypertension in the third trimester is a particularly morbid event. Outcomes are worse than those seen with chronic vasoconstriction. We find this event to be an important clinical marker for the need for increased fetal surveillance.

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Magnesium sulfate versus phenytoin for seizure prophylaxis in pregnancy-induced hypertension

Margaret P. Appleton, MD, Thomas J. Kuehl, PhD, Marsha A. Raebel, PharmD, H. Ray Adams, PhD, Alfred B. Knight, MD, and William R. Gold, MD Temple, Texas

Seizure prophylaxis is standard intrapartum therapy for patients with pregnancy-induced hypertension. Magnesium sulfate is used in the United States in spite of limited literature comparing its efficacy with other anticonvulsants. Fifty patients with pregnancy-induced hypertension were prospectively randomized to receive magnesium sulfate or phenytoin for seizure prophylaxis. Patients were observed for toxicity, side effects, and labor outcomes, and the neonates were evaluated for side effects of the therapy. Three patients were excluded with adverse reactions to medications (one in magnesium sulfate group, two in phenytoin group). No differences were found in patient tolerance, adverse reactions, or neonatal outcomes between groups. Maternal free phenytoin levels were 13.0% ± 0.4% of total phenytoin (serum albumin, 2.5 to 3.5 gm/dl), significantly higher than in nonpregnant patients. Neither free phenytoin levels nor percentage of total phenytoin that was free correlated significantly with maternal albumin levels. The pharmacokinetics of phenytoin loading in the massively obese pregnant patient may differ and require further evaluation. Phenytoin is a well-tolerated alternative to magnesium sulfate for seizure prophylaxis in the patient with mild pregnancy-induced hypertension. (AM J OBSTET GYNECOL 1991;165:907-13.)

Key words: Pregnancy-induced hypertension, seizure prophylaxis, magnesium sulfate, phenytoin

Magnesium sulfate is the standard of care in the United States for seizure prophylaxis in pregnancyinduced hypertension,1 although its efficacy has not been substantiated when compared with other anticonvulsant agents such as phenytoin.24 Recent studies have addressed phenytoin pharmacokinetics and efficacy in patients with pregnancy-induced hypertension. 5-9 The following hypotheses were proposed to evaluate other aspects of therapy in 50 patients prospectively randomized to magnesium sulfate or phenytoin.

- 1. The safety of phenytoin, as defined by intrapartum complications and adverse reactions, will compare favorably with magnesium sulfate in the term and near-term pregnant patient with pregnancy-induced hypertension.
- 2. There will be better patient tolerance of side effects with phenytoin compared with magnesium sulfate as demonstrated by patient and nursing questionnaires.
- 3. Neonatal outcome will not differ in the two groups.

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Therapeutic phenytoin levels are a function of the percentage of phenytoin not bound (free phenytoin).6 Generally, 90% of plasma phenytoin is present as an inactive form bound to albumin. The phenytoin level measured clinically is total (bound and free) phenytoin. Because albumin levels are decreased in pregnancy and in pregnancy-induced hypertension, total phenytoin levels may not reflect the ratio of free to total phenytoin, and therefore a different therapeutic range for total phenytoin has been proposed.6 Serum free and total

phenytoin, albumin, and total protein concentrations

were measured to further evaluate this concept.

Material and methods

Subjects. From November 1989 to November 1990, 50 patients with pregnancy-induced hypertension, at ≥36 weeks' gestation, were prospectively randomized to prophylaxis with magnesium sulfate or phenytoin. Eleven patients met criteria for severe pregnancy-induced hypertension, and one had an eclamptic seizure before admission. The remaining had mild pregnancyinduced hypertension. Laboratory monitoring included urinalysis, complete blood cell count, platelet count, liver enzymes, and clotting parameters.

Protocol for magnesium sulfate. The 4 gm loading dose (100 ml of a solution containing 40 gm magnesium sulfate diluted in 1000 ml 5% dextrose in water) was infused intravenously over 20 minutes. The maintenance dosage was initiated by continuous infusion at 2 gm/hr (50 ml/hr of same solution) and was adjusted

Table I. Comparison* of patient and pregnancy characteristics between groups

Characteristic	Magnesium sulfate group	Phenytoin group			
No. of patients	24	23			
Maternal age (yr)†	23 ± 5	25 ± 6			
Maternal weight (lb)†	182 ± 32	191 ± 54			
Maternal ethnic group (white/black/Hispanic/ Oriental	17/5/1/1	10/7/6/0			
Nulliparity	16/24	13/23			
Fetal sex (male/female)	17/7	15/8			
Severity of pregnancy- induced hypertension (severe/mild)	5/19	6/17			
Initial blood pressure					
Systolic (mm Hg)†	149 ± 10	154 ± 22			
Diastolic (mm Hg)†	90 ± 7	93 ± 10			
Highest blood pressure					
Systolic (mm Hg)†	170 ± 13	170 ± 22			
Diastolic (mm Hg)†	100 ± 10	100 ± 10			
Initial cervical examination					
Dilatation (cm)†	2.0 ± 1.4	2.1 ± 1.5			
Effacement (%)	62 ± 29	50 ± 37			
Station	-3.1 ± 1.1	-3.0 ± 1.0			
Induced/spontaneous	18/6	16/7			

*No significant differences between groups with χ^2 analysis to test characteristics expressed as proportions, Student t test or analysis of variance to test characteristics expressed as continuous variables, and Mann-Whitney U test to test variables expressed as discontinuous variables.

†Results expressed as mean ± SD.

to serum levels between 4 and 7 mg/dl. Serum Mg²⁺ levels were measured every 6 hours.

Protocol for phenytoin. An initial loading dose of 10 mg/kg was infused intravenously at a rate of 25 to 40 mg/min, followed 2 hours later by an additional dose of 5 mg/kg as per Ryan et al.⁶ Blood pressure and pulse were recorded every 2 minutes during infusion and every 15 minutes thereafter. Continuous electrocardiac monitoring was used during infusion only. A maintenance dose of 200 mg was initiated 12 hours after the second loading dose and repeated every 8 hours. This dose was given intravenously during the intrapartum period and orally post partum for 24 hours and was then discontinued. Free and total serum phenytoin levels, albumin, and total protein were measured 8 and 14 hours after the initial loading dose.

Common aspects of both protocols. All patients were cared for in our intensive observation unit for 24 hours post partum, were given nothing by mouth, had vital signs taken every 15 minutes, had hourly intake and output checks, and had indwelling bladder catheters. Twenty-four hours post partum the seizure prophylaxis was discontinued, and the patients were transferred to the postpartum unit.

Patients and nurses were asked to complete a questionnaire between 24 and 36 hours post partum. Each was asked to rank (between 1 and 5) the following char-

acteristics by severity, with low numbers representing little or no problem and high numbers severe or constant problem: nausea or vomiting, blurred or double vision, drowsiness, and difficulty speaking. Apgar scores, umbilical cord arterial and venous blood gas values, and neurologic evaluation were obtained on all neonates. Magnesium sulfate, total and free phenytoin, and total protein and albumin concentrations were determined in umbilical cord serum for the appropriate groups.

Phenytoin and plasma protein assays. Serum protein and albumin were assayed by standard methods, and total phenytoin was assayed on an Abbott TDX (Abbott Laboratories, Chicago) by fluorescence polarization immunoassay. Free phenytoin levels were determined in batches of samples after ultrafiltration to remove plasma proteins using the technique described by Oellerich and Muller-Vahl. 10, 11

Informed consent. The study was approved by the Institutional Review Board of Scott and White Memorial Hospital. Informed consent was obtained from each patient before enrollment in the protocol.

Statistical methods. Groups were compared with statistical techniques, including analysis of variance and the Student t test, for characteristics expressed as continuous variables, χ^2 analysis to test characteristics expressed as proportions, and the Mann-Whitney U test to test characteristics expressed as noncontinuous or nonparametric variables. Comparisons were also made with multifactorial or single-factor analysis of variance when appropriate. Software used included PC Statistician and PC ANOVA (Human Systems Dynamics, Northridge, Calif.).

Results

Fifty patients were randomized by ordered packets to two groups, magnesium sulfate or phenytoin. Three patients were excluded. The remaining patients (24 in magnesium sulfate group and 23 in phenytoin group) completed the protocol. No differences were evident between the antepartum clinical characteristics of the two groups (Table I). Intrapartum characteristics (Table II) demonstrate no significant differences in incidence of cesarean section, dose-to-delivery intervals, and duration of second stage of labor. A difference of 2.5 hours between the lengths of first stage of labor was observed, but this did not reach statistical significance. The length of hospitalization for vaginally delivered phenytoin patients was also shorter than that for the magnesium sulfate patients, but this difference was not statistically significant (p = 0.06).

Both protocols were safe and well tolerated. No seizures occurred in either group. Three patients were excluded for adverse reactions; one patient became extremely anxious 30 seconds after starting the magne-

Magnesium sulfate-treated Phenytoin-treated Characteristic Vaginal delivery Cesarean section Vaginal delivery Cesarean section 6 5 No. of patients 18 18 12 No. nulliparous 11 23.8 9.3 15.6 Dose-to-delivery interval (hr) 15.1 Length of first stage of labor (hr)† 13.8 11.3 Length of second stage of labor (hr)† 1.02 0.94Length of hospital stay (days)‡ 4.7 4.4

Table II. Comparison* of magnesium sulfate- and phenytoin-treated patients

3.3

sium sulfate loading dose and demanded discontinuance of the drug. Two patients were excluded from the phenytoin group, one for itching (without rash) that occurred after the second loading dose and the other for symptomatic hypotension (blood pressure from 200/100 to 120/60 mm Hg) and dysphoria that occurred during initial phenytoin infusion. The latter patient had a phenytoin level of 13.4 µg/ml, well within the therapeutic range. This reaction may have been ameliorated by slowing the infusion rate further to <25 mg/min. Each of these patients received the other drug during labor without sequelae and were excluded from further evaluation. Burning at the intravenous injection site was noted by one other patient receiving phenytoin.

Patients and their nurses reported mild side effects (Table III) with no differences between mean or total scores for the four characteristics. The nursing and patient scores correlated well.

No neonatal adverse outcomes were documented. Apgar scores (1 min, 7.4 vs 7.8; 5 min, 8.9 vs 9.0), umbilical cord blood gases (artery, 7.26 ± 0.06 vs 7.25 ± 0.03), and birth weights (3110 ± 615 vs 3122 ± 602 gm) were not different between groups and were normal. The initial and discharge physical examinations, including the neurologic examination, were within normal limits. All babies went home with their mothers (one baby with intrauterine growth retardation was observed two days), and length of hospitalization was not different in the two groups (3.1 ± 0.4) days for magnesium sulfate infants and 2.5 ± 0.3 days for phenytoin infants). There was a preponderance of male neonates in our study (32 male, 15 female).

Magnesium sulfate levels were therapeutic at initial evaluation in 22 of 24 patients. Neonatal levels paralleled maternal levels (Fig. 1).

Twenty-two of 23 patients receiving phenytoin had free levels between 1 and 2 µg/ml, considered to be the therapeutic range. Neonatal free and total phenytoin levels are shown by intervals from first dose (Fig. 2). With the nonpregnant total phenytoin therapeutic range (10 to 20 μg/ml), nine of 27 maternal samples would have been considered subtherapeutic. Serum albumin concentrations were very similar within the three groups of samples (two maternal and one neonatal group) but differed from each other and correlated to percent free phenytoin (Fig. 3). The correlation coefficient was low ($R^2 = 0.36$; percent free phenytoin = 19.9 - 2.29 [albumin]), suggesting that low serum albumin explains only a small proportion (36%) of the increase in free phenytoin. In the two maternal samples free phenytoin (mean ± SE) was 13.1% ± 0.4% (range, 9.5% to 16.0%) and $13.9\% \pm 0.5\%$ (range, 9.2% to 14.3%), significantly greater than in neonatal samples, $10.2\% \pm 0.7\%$ (range, 9.2% to 14.2%).

2.6

One patient had levels above the normal therapeutic range for both total and free (21.1 and 2.8 µg/ml, respectively); neonatal levels reflected these high maternal levels (total, 23.8 µg/ml; free, 2.6 µg/ml). The total loading dose, 2400 mg, was calculated from the patient's weight (>350 pounds). Neither the patient nor her neonate had clinical symptoms of phenytoin tox-

Comment

Most patients with pregnancy-induced hypertension do not progress to seizures. However, identification of the subgroup of patients who are at greatest risks for seizures (for example, by providing prophylaxis for only those patients with severe pregnancy-induced hypertension or hyperreflexia) may increase specificity but not sensitivity. Hence, at our institution all patients who meet clinical criteria for pregnancy-induced hypertension receive intrapartum seizure prophylaxis.

Both magnesium sulfate and phenytoin are associated with risks and side effects. 6, 12 Magnesium sulfate can produce nausea, blurred vision, drowsiness, and depression of the deep tendon reflexes at therapeutic

^{*}Comparisons made with multifactorial or single-factor analysis of variance. There were no significant interactions between type of treatment and mode of delivery.

[†]Comparisons of nulliparous patients delivered vaginally made with single-factor analysis of variance. No significant differences. ‡Only significant difference in this series of comparisons was for the effect of mode of delivery on length of hospital stay (p = 0.011).

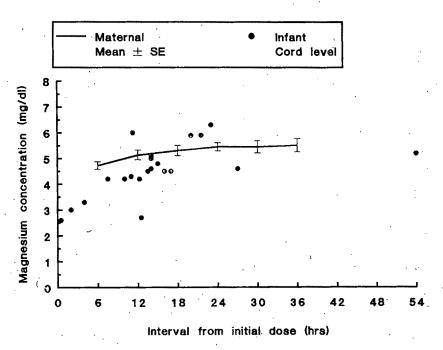


Fig. 1. Serum magnesium concentrations (milligrams per deciliter) for individual newborns (●) and mothers (mean ± SE) plotted (in hours) from initial loading dose.

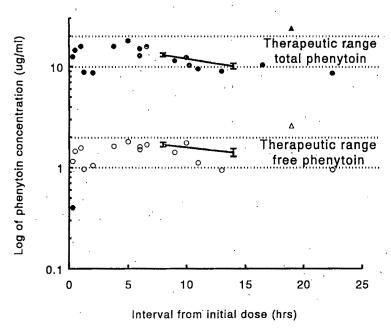


Fig. 2. Serum total (\bullet) and free (\circ) phenytoin levels (micrograms per milliliter) for individual newborns and mothers (mean \pm SE) plotted (in hours) from first loading dose. Single infant of patient with maternal weight >350 pounds (\triangle and \triangle). Note log concentration on y axis.

levels (4 to 7 mg/dl). At plasma levels above 10 mg/dl respiratory depression can occur. The patient who was reported to have an adverse reaction on magnesium sulfate likely had an emotional reaction to medication rather than a true toxic response.

The side effects of phenytoin during short-term use are also dose-dependent and have been mild in other studies. When serum total phenytoin levels exceed 20 $\mu g/ml$, a significant risk of nystagmus occurs. At 30 $\mu g/ml$ ataxia and incoordination are observed. Because we have had limited experience with intravenous phenytoin, we chose to be particularly cautious. The first patient excluded from the phenytoin group had diffuse itching without a rash. This reaction may have been

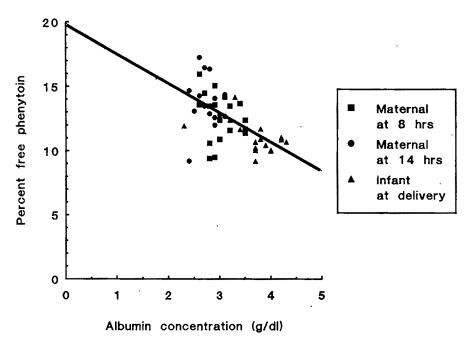


Fig. 3. Percentage free phenytoin versus albumin levels (grams per deciliter) in first maternal sample (\blacksquare), second maternal sample (\bullet), and neonatal sample (\triangle). Regression coefficient r=0.6.

Table III. Comparison* of side effects on the basis of responses to questionnaire by patients and nurses

	Magnesium s	ulfate-ireated	Phenytoin-treated	
Side effect	Patient	Nurse	Patient	Nurse
Intrapartum (No. responding)	14	16	16	19
Nausea or vomiting	2.0	1.8	1.8	1.4
Blurred vision	1.7	1.4	1.3	1.4
Drowsiness	2.7	2.6	2.6	2.3
Difficulty speaking clearly	1.9	1.6	1.8	1.4
Total score	8.4	7.6	7.1	6.5
Post partum (No. responding)	14	16	. 17	19
Nausea or vomiting	1.2	1.4	1.4	1.1
Blurred vision	1.9	1.2	1.4	1.3
Drowsiness	3.0	2.4	2.9	2.5
Total score	6.1	4.9	5.7	4.8

^{*}No sigificant differences between groups for either patient or nurse responses to questions about side effects. Mann-Whitney U test used for group comparisons.

related to the coincidental placement of a bupivacaine hydrochloride and fentanyl epidural block. The second patient excluded from the phenytoin group had a known phenytoin reaction (hypotension) that can be appropriately treated by slowing the rate of infusion. No patient's outcome was altered by these reactions.

We had anticipated that the side effects of phenytoin would be significantly fewer than those of magnesium sulfate. Both regimens were tolerated well, as documented by our patients and their nurses. For the purpose of this study, patients receiving phenytoin were not managed differently from patients receiving magnesium sulfate. Much of the intensive monitoring of mild pregnancy-induced hypertension in postpartum patients is required because of the potential side effects

of magnesium sulfate. This monitoring protocol could be simplified if the patient were on phenytoin. Indeed, even the continuous electrocardiographic monitoring may not be necessary during phenytoin loading doses in mild pregnancy-induced hypertension in patients without cardiac disease. Our study suggests a shorter length of hospitalization in phenytoin-treated patients delivered vaginally. Our impression is that phenytointreated patients felt better more quickly and were likely to be discharged earlier. However, length of hospitalization was not a part of the study hypotheses, and therefore variables relating to length of stay were not controlled.

No adverse effects were noted in the neonates. Toxic effects of magnesium sulfate on the neonate have been suggested, postulated, and in general, not supported. Phenytoin is used in neonates to treat seizures with few complications.¹⁸

No patient in either group had seizures after the initiation of prophylaxis. However, the small number of patients in our study does not allow an assessment of efficacy for seizure prevention. Dommisse8 has challenged phenytoin's ability to prevent recurrence of seizures in eclamptic patients. Four of 11 of his patients treated with phenytoin had recurrent seizures in spite of therapeutic levels of total phenytoin, whereas none of the 11 patients on magnesium sulfate had recurrent seizures. Free phenytoin levels were not measured. Ten percent to fifteen percent of eclamptic patients will have recurrent seizures in spite of therapeutic anticonvulsant levels,12 and many eclamptic patients have central nervous system pathology that would predispose them to repetitive seizures.14 Persistent seizures after treatment with phenytoin has recently been reported by Coyaji and Otiv.9 Ryan15 has proposed a multicentered study to address the efficacy of seizure prophylaxis in a response to Dommisse's report. Indeed, phenytoin may offer less advantage to the patient with severe pregnancy-induced hypertension or the eclamptic patient. Intensive monitoring of these patients for at least 24 hours post partum would be necessary, independent of the anticonvulsant used.

For most drugs the rate of metabolism is directly proportional to the plasma concentration, i.e., the pharmacokinetics are first-order. This is not true for phenytoin, a drug that has capacity-limited or saturable metabolism. For most patients the rate of phenytoin metabolism at therapeutic plasma concentrations is close to the limit. Small dosage adjustments within the therapeutic range can lead to disproportionate increases in both plasma total and free phenytoin concentrations. Saturation of the enzyme systems responsible for phenytoin metabolism is often the cause of phenytoin toxicity; careful attention to phenytoin dosing is therefore appropriate.

There was not a wide variance in albumin or total protein levels among our patients. A concern that the maternal low protein levels (specifically albumin) would increase the percentage of free phenytoin and thus increase toxicity was not substantiated. Ryan et al.⁶ proposed a different therapeutic range for total phenytoin during pregnancy, 7 to 17 µg/ml (nonpregnant, 10 to 20 µg/ml), on the basis of the decrease in albumin levels. Their therapeutic ranges are derived from a formula that adjusts the total phenytoin for the patients' albumin levels. Neither their formula nor others in their reference¹⁶ accurately estimate free phenytoin levels in our patient population. Beck et al.¹⁷ attempted to estimate free phenytoin levels with multiple serum biochemical and clinical parameters. Serum albumin

level was the primary variable, but it alone explained at best 77% of the observed free phenytoin variability $(C_F = 1.53 + [0.09 \ C_T] - 0.43$ albumin, where C_F is free phenytoin and C_T is total phenytoin clearance). Factors known to affect binding of phenytoin to albumin include liver disease, renal disease (elevated blood urea nitrogen), elevated liver enzymes (specifically aspartate aminotransferase), and elevated bilirubin. An increased free fatty acids level may alter the percentage of free phenytoin. Free fatty acid levels were not measured in our patients.

This study confirms the work of Ryan et al.,6 in which a two-phase loading dose of phenytoin was proposed (range of our loading doses was 675 to 1930 mg, excluding the patient mentioned below). Free phenytoin levels were therapeutic and relatively independent of total phenytoin levels. The single exception was a morbidly obese young woman whose loading dose was 2400 mg; she had no clinical symptoms suggestive of a toxic dose. It is possible that the high plasma levels observed in our morbidly obese patient reflect a patient with a low metabolic capacity for phenytoin and not solely an obesity effect. A maximum loading dose of 2100 mg is suggested until larger studies can address the additional problem of the pregnant obese patient.

In our study no alteration in dosing was made on the basis of total phenytoin concentration. Whether monitoring of free phenytoin levels or total levels by using an adjusted therapeutic range is appropriate cannot be decided by this study. Phenytoin is commonly used to treat seizure disorders during pregnancy. The dose of phenytoin is often increased on the basis of the lower serum total levels found during pregnancy. Our study suggests that the free component of phenytoin is likely still in the therapeutic range and that increasing the dose is unnecessary.

In summary, this study confirms that both magnesium sulfate and phenytoin, when used for seizure prophylaxis, have limited side effects and are well tolerated by patients and neonates. Patients with mild pregnancyinduced hypertension (at least 70% of our patients) who are at low risk for seizures could benefit from the reduced intensity of monitoring acceptable with intravenous phenytoin therapy. The comparative efficacy of seizure prophylaxis with phenytoin or magnesium sulfate or simply expectant management of patients with pregnancy-induced hypertension must await a randomized, multicentered clinical trial. To determine the size of a proposed trial, we estimate the incidence of eclampsia to be approximately 1% of patients with pregnancy-induced hypertension; half have seizures before receiving medical care. To detect a 50% change in seizure incidence ($\alpha = 0.05$, power = 0.8), a patient population of about 9100 would be required. The patient who has seizures before admission or who has

severe pregnancy-induced hypertension may constitute a unique subgroup of patients and should be evaluated separately in any proposed trial.

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Editors' note

The American Journal of Obstetrics and Gynecology introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case reports, and brief communications articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Incidence and risk factors associated with abnormal postpartum glucose tolerance in women with gestational diabetes

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To determine the incidence and risk factors associated with an abnormal postpartum glucose tolerance in women with gestational diabetes, 103 patients with gestational diabetes had a 2-hour, 75 gm oral glucose tolerance test 6 ± 2 weeks (mean \pm SD) after delivery. Twenty-two percent (23/103) of results were abnormal: Three showed frank diabetes, four showed impaired glucose tolerance, and 16 were nondiagnostic. There was a significant difference in gravidity, pregravid weight and body mass index, delivery weight, gestational age at diagnosis, fasting and 2- and 3-hour glucose level at the time of the oral glucose tolerance test during pregnancy, need for insulin therapy during gestation, and neonatal weight >4000 gm in the abnormal group as compared with the normal group. Elevated fasting glucose level (p = 0.0001) and earlier gestational age at time of diagnosis of gestational diabetes (p = 0.013) were found to be most predictive of an abnormal postpartum glucose tolerance test result. These results support the importance of postpartum oral glucose tolerance testing in women with gestational diabetes. (AM J OBSTET GYNECOL 1991;165:914-9.)

Key words: Postpartum glucose tolerance, gestational diabetes

Gestational diabetes is the most common metabolic abnormality affecting pregnant women. Approximately 3% of all pregnant women have gestational diabetes, representing 80% of all pregnancies complicated by diabetes mellitus. Women with gestational diabetes have an increased risk for developing diabetes mellitus in later life. The prevalence of diabetes in the 15-year follow-up studies of O'Sullivan³ ranged between 25% and 50% in lean and obese women with gestational diabetes, whereas it was only 2% and 4% in lean and obese women with normal glucose tolerance during gestation. Others have also found an increased risk of overt diabetes mellitus in different populations in later life. 4.5

On the basis of these findings, the Second International Workshop-Conference on Gestational Diabetes⁶ has recommended that all women in whom a diagnosis of gestational diabetes was made should be evaluated

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at the first postpartum visit by a 2-hour, 75 gm oral glucose tolerance test (GTT) in accordance with the criteria of the National Diabetes Data Group or World Health Organization. The only study to date that has examined the outcome of this recommendation was the recent study by Kjos et al. They found a 19% (48/246) incidence of abnormal oral glucose tolerance in the early postpartum period in a primarily Hispanic population (98%) of women with gestational diabetes. Hence, the purpose of our study was to determine the incidence of abnormal postpartum glucose tolerance in women with gestational diabetes in our population and to evaluate which risk factors are predictive of an abnormal result.

Methods

Subjects. This retrospective study was carried out at MetroHealth Medical Center, Cleveland, and the Medical Center Hospital of Vermont, Burlington. Maternal and neonatal records from patients with gestational diabetes from 1987 through 1989 were reviewed. Most of the patients received their primary obstetric care at these hospitals, although some patients were referred from outlying physicians to the Maternal-Fetal Medicine service. All obstetric patients were screened for gestational diabetes using a 50 gm, 1-hour oral glucose load between 24 and 28 weeks' gestation. Women with risk factors for gestational diabetes (previous gestational diabetes, history of fetal macrosomia, prior still-birth, persistent glucosuria, maternal obesity, and fam-

ily history of type II diabetes mellitus) were screened at their initial visit and again at 24 to 28 weeks' gestation. Patients with a venous plasma glucose value of >135 mg/dl underwent a 100 gm, 3-hour oral GTT. All patients were instructed to follow a diet that provided at least 150 gm carbohydrate for 3 days and to fast for at least 10 hours before the GTT. The diagnosis of gestational diabetes was made with the criteria of Carpenter and Coustan,8 i.e., two or more of these venous plasma glucose concentrations were met or exceeded: fasting, 95 mg/dl; 1 hour, 180 mg/dl; 2 hours, 155 mg/dl; and 3 hours, 140 mg/dl.

All patients with gestational diabetes were instructed in diet therapy by a registered dietician. The diet consisted of 30 to 35 kcal/kg ideal body weight, 50% of the calories as complex carbohydrates and the remaining calories as fat, 80 to 100 gm protein, and 20 gm dietary fiber. Women with gestational diabetes either performed home blood glucose monitoring or had weekly fasting and postprandial glucose determinations in the pregnancy diabetic clinic. Insulin therapy was instituted if fasting or preprandial glucose determinations were consistently >100 mg/dl or 2-hour postprandial glucose determinations were >120 mg/dl. None of the patients were started on prophylactic insulin therapy. The characterization of neonatal birth weight as small for gestational age (SGA, <10th percentile), average for gestational age (AGA, between 10th and 90th percentiles), and large for gestational age (LGA, >90th percentile) was based on normative birth weight curves for gestational age developed at each hospital by one of the authors (P.M.C.). Maternal estimates of obesity were described with the body mass index: Weight (kilograms)/Height (meters)2.

All patients with gestational diabetes were urged to have a 2-hour, 75 gm oral GTT at the time of the postpartum visit. They were again instructed to consume ≥150 gm carbohydrate per day for 3 days and fast for at least 10 hours before the GTT. Plasma glucose values were obtained in the fasting state and then at 30, 60, 90, and 120 minutes after the glucose ingestion. Glucose concentrations were measured with the glucose oxidase method in the hospital or Clinical Research Center laboratory. Postpartum oral GTTs were interpreted according to the criteria of the National Diabetes Data group.9 The diagnosis of diabetes mellitus in nonpregnant adults is made either by an elevated venous plasma glucose level >140 mg/dl or by the 2-hour venous plasma glucose sample and any one of the 30-, 60-, or 90-minute venous plasma glucose samples >200 mg/dl. Three criteria are needed for the diagnosis of impaired glucose tolerance: (1) fasting venous plasma glucose level <140 mg/dl, (2) venous plasma glucose concentration at 30, 60, or 90 minutes >200 mg/dl, and (3) 2-hour venous plasma glucose concentration between 140 and 200 mg/dl. Normal glucose tolerance is defined as (1) fasting venous plasma glucose level <115 mg/dl, (2) 30-, 60-, and 90-minute venous plasma glucose concentrations <200 mg/dl, and (3) 2-hour venous plasma glucose concentration <140 mg/dl. Venous plasma glucose concentrations above those defined for normal glucose tolerance but below the criteria for impaired glucose tolerance are considered nondiagnostic.

Statistical analysis. The data are expressed as mean ± SD. The difference between groups was determined by unpaired Student t test, two-sample Wilcoxon test, χ^2 analysis, or generalized Fisher's exact test. Stepwise logistic regression analysis was used to determine which risk factors were most predictive of an abnormal postpartum GTT. Statistical analyses were performed with a BMDP statistical software package. Probability levels of ≤0.05 were considered significant.

Results

We were able to review 115 maternal and neonatal records of women who had gestational diabetes and a 2-hour, 75-gm GTT at their postpartum visit. Twelve patients were excluded from analysis because of incomplete records. Thus 103 patient records were included in the final analysis. Of these, 46 were from the Case Western Reserve University and 57 were from the University of Vermont.

Our study population consisted of 82% white, 6% black, 6% Hispanic, and 6% other (Asian, Middle Eastern, mixed) patients. Forty-nine percent of these women had a positive family history of diabetes mellitus, and 20% had a previous history of gestational diabetes. Fifty-one women (49%) required insulin therapy for glucose control at some time during their pregnancy.

The postpartum GTTs were completed at 6.6 ± 1.6 weeks after delivery. Forty-four (43%) of women were breast-feeding, and nine (9%) were using oral contraceptives at the time of the postpartum GTT. Twentythree (22%) of the 75 gm, 2-hour GTTs were abnormal: three, frank diabetes mellitus; four, impaired glucose tolerance, and 16, nondiagnostic.

We next compared the maternal and neonatal characteristics of the patients with normal and abnormal postpartum glucose tolerance (Table I). There was a significant difference in gravidity $(3.7 \pm 2.4 \text{ vs})$ 2.8 ± 1.9 ; p = 0.05), pregravid weight (84.6 ± 18.3 vs $71.8 \pm 20.0 \,\mathrm{kg}$; p = 0.002), pregravid body mass index $(32.5 \pm 6.8 \text{ vs } 27.2 \pm 7.6 \text{ kg/m}^2; p = 0.003)$, and delivery weight $(97.6 \pm 16.3 \text{ vs } 84.3 \pm 17.0 \text{ kg})$ p = 0.001) in women with abnormal postpartum glucose tolerance as compared with the normal group. Gestational age at the time of diagnosis of gestational diabetes (22.4 \pm 7.7 vs 27.7 \pm 6.1 weeks' gestation; p = 0.0009) was significantly less in the abnormal post-

Table I. Maternal and neonatal characteristics of 103 patients with normal (n = 80) and abnormal (n = 23) glucose tolerance at postpartum visit

	Normal	Abnormal	p Value
Maternal age (yr)	29.3 ± 5.6	30.4 ± 5.5	0.40
Gravidity	2.8 ± 1.9	3.7 ± 2.4	0.05
Parity	1.0 ± 1.5	1.6 ± 1.9	0.11
Pregravid features			•
Weight (kg)	71.8 ± 20.0	84.6 ± 18.3	0.002
Height (cm)	162.5 ± 0.1	163.0 ± 0.1	0.68
Body mass index (kg/m²)	27.2 ± 7.6	32.5 ± 6.8	0.003
Delivery weight (kg)	84.3 ± 17.0	97.6 ± 16.3	0.001
Maternal weight gain (kg)	12.2 ± 6.3	11.2 ± 6.6	0.51
Maternal weight gain as % of pregravid weight	19.4 ± 11.9	14.3 ± 10.5	0.07
Gestational age at diagnosis (wks)	27.7 ± 6.1	22.4 ± 7.7	0.0009
Neonatal			
Birth weight (gm)	3414 ± 540	3478 ± 843	0.66
Length (cm)	50.4 ± 2.9	51.0 ± 3.6	0.39
Estimated gestational age at delivery (wk)	38.4 ± 1.5	37.9 ± 2.5	0.20
Incidence of SGA, AGA,	,		
LGA neonates	•		
SGA (<10%)	2 (2.6%)	0 (0%)	
AGA (10-90%)	54 (69.2%)	15 (65.2%) }	0.64
LGA (>90%)	22 (28.2%)	8 (34.8%)	
Neonatal weight >4000 gm	9 (11.4%)	7 (30.4%)	0.03
Neonatal sex			•
Male	45 (57%)	15 (61%)	0.00
Female	34 (42%)	9 (39%)	0.69

Table II. Maternal data relating to glucose tolerance in 103 patients with normal (n = 80) and abnormal (n = 23) postpartum glucose tolerance

•	Normal	Abnormal	p Value
Positive family history of diabetes mellitus	44 (57%)	15 (65%)	0.65
Previous gestational diabetes	14 (17%)	7 (30%)	0.29
3 hr, 100 gm GTT (mg/dl)			
Fasting	96.5 ± 13.2	117.2 ± 26.1	0.0001
I hr	206.5 ± 23.6	216.6 ± 60.0	0.23
2 hr	185.0 ± 30.1	206.2 ± 51.1	0.015
3 hr	128.2 ± 33.2	154.7 ± 56.1	0.007
Insulin therapy during gestation	32 (40%)	18 (78%)	0.003
Breast-feeding at time of postpartum oral GTT	35 (44%)	8 (36%)	0.67
Oral contraceptives at time of postpartum oral GTT	8 (10%)	1 (5%)	0.67

partum glucose tolerance group as compared with the normal group. Although approximately 30% of the neonates in each group were LGA, there were significantly more infants (7/23, 30%, vs 9/80, 11%; p = 0.03) of mothers with gestational diabetes who were >4000 gm in the abnormal postpartum glucose tolerance group as compared with the normal group. There were more male than female neonates of these woman (60 male vs 43 female; p = 0.09); however, this did not reach statistical significance.

The maternal data relating to glucose tolerance are

given in Table II. The fasting (117.4 \pm 26.1 vs 96.5 \pm 13.2 mg/dl; p=0.0001), 2-hour (206.2 \pm 51.1 vs 185.0 \pm 30.1 mg/dl; p=0.015), and 3-hour (154.7 \pm 56.1 vs 128.2 + 33.2 mg/dl; p=0.007) glucose concentrations during gestation were significantly greater in the abnormal postpartum GTT group than in the normal group. Furthermore, 18 (78%) of the patients with abnormal postpartum glucose tolerance required insulin therapy during gestation as compared with 32 (40%) of the patients with normal postpartum glucose tolerance (p=0.003).

Table III. Maternal and neonatal characteristics of 87 patients with normal glucose tolerance (n = 80) and frank glucose intolerance (n = 7) post partum

		Normal glucose tolerance	Frank glucose intolerance	p Value
Maternal age (yr)	, *	29.3 ± 5.6	33.7 ± 3.0	0.03
Gravidity		2.8 ± 1.9	4.7 ± 2.9	0.04
Parity -		1.0 ± 1.5	2.1 ± 2.1	0.10
Pregravidity features		• • • •		
Weight (kg)		71.8 ± 20.0	86.0 ± 20.1	0.05
Height (cm)		162.5 ± 0.1	164.0 ± 0.1	0.62
Body mass index		27.2 ± 7.6	32.2 ± 8.1	0.08
(kg/m^2)			•	,
Delivery weight (kg)	• •	84.3 ± 17.0	96.7 ± 21.3	0.09
Maternal weight gain (kg)		12.2 ± 6.3	10.6 ± 4.2	0.37
Maternal weight gain as % of pregravid weight	*	19.4 ± 11.9	12.6 ± 5.0	0.08
Gestational age at diagnosis of gestational diabetes (wk)		27.7 ± 6.1	19.0 ± 9.2	0.006
Neonatal			•	
Birth weight (gm)	•	3414 ± 540	3463 ± 1198	0.16
Length (cm)	. •	50.4 ± 2.9	51.4 ± 2.4	0.57
Estimated gestational age at		38.4 ± 1.5	36.4 ± 3.8	0.17
delivery (wk)				
Incidence of SGA, AGA,			* 4	
LGA neonates			***	
SGA (<10%)	*	2 (2.6%)	0 (0%)	
AGA (10%-90%)		54 (69.2%)	4 (57%) }	0.55
LGA (>90%)	•	22 (28.2%)	3 (43%)	
Neonatal weight >4000 gm		9 (11%)	3 (43%)	0.05
Neonatal sex		• • • • • • • • • • • • • • • • • • • •	,	•
Male	. : ,	45 (57%)	5 (71%)	0.69
Female		34 (43%)	2 (28%)	

With the use of stepwise logistic regression analysis adjusted for any possible location difference, fasting glucose level at the time of the oral GTT (p = 0.0001) during gestation and gestational age at the time of the abnormal test (p = 0.013) were the best predictors of abnormal postpartum glucose tolerance in these women. Although not significant, the next best predictor of abnormal postpartum glucose tolerance was insulin therapy during gestation (p = 0.08).

Because 16 of the patients in the abnormal group had a nondiagnostic postpartum GTT result, we further analyzed the data comparing the seven women with frank glucose intolerance (three with diabetes mellitus, four with impaired glucose tolerance) with the 80 women with normal postpartum glucose tolerance. The results are given in Tables III and IV. Maternal age $(33.7 \pm 3.0 \text{ vs } 29.3 \pm 5.6 \text{ years; } p = 0.03)$, gravidity $(4.7 \pm 2.9 \text{ vs } 2.8 \pm 1.9; p = 0.04)$, and pregravid weight (86.0 \pm 20.1 vs 71.8 \pm 20.0 kg; p = 0.05) were significantly greater in the women with frank glucose intolerance than in the women with normal postpartum glucose tolerance. Estimated gestational age at the time of diagnosis of gestational diabetes was again earlier and highly significant in the women with frank glucose intolerance (19.0 \pm 9.2 vs 27.7 \pm 6.1 weeks' gestation; p = 0.006) as compared with the other group. Although estimated gestational age at the time of delivery was earlier in the women with frank glucose intolerance $(36.4 \pm 3.8 \text{ vs } 38.4 \pm 1.5 \text{ weeks' gestation; } p = 0.17),$ the incidence of neonatal weight >4000 gm was significantly greater (3/7, 43%, vs 9/80, 11%; p = 0.05) in these seven patients as compared with the women with normal postpartum glucose tolerance.

The maternal data relating to glucose tolerance in these 87 patients are given in Table IV. The fasting $(136.6 \pm 24.8 \text{ vs } 96.5 \pm 13.2 \text{ mg/dl}; p = 0.0001),$ 1-hour $(250.4 \pm 36.6 \text{ vs } 206.5 \pm 23.6 \text{ mg/dl}; p =$ 0.001), 2-hour (224.3 \pm 73.2 vs 185.0 \pm 30.1 mg/dl; p = 0.03), and 3-hour (200.3 ± 68.2 vs 128.2 ± 33.2 mg/dl; p = 0.005) glucose concentrations during gestation were significantly greater in the patients with frank glucose intolerance as compared with the normal postpartum GTT group. Furthermore, all seven (100%) of the patients with frank glucose intolerance required insulin therapy during gestation as compared with 32 (40%) of the patients with normal postpartum glucose tolerance (p = 0.003). Stepwise logistic regression analysis was not used to determine which risk factors were most predictive of postpartum frank glucose intolerance because of the small number of subjects in this group.

Comment

The results of this study demonstrate that ar mately 20% of women with gestational diabeter have normal postpartum glucose tolerance

Table IV. Maternal data relating to glucose tolerance in 87 patients with normal glucose tolerance (n = 80) and frank glucose intolerance (n = 8) post partum

	Normal glucose tolerance	Frank glucose intolerance	p Value
Positive family history of diabetes mellitus	44 (57%)	4 (57%)	1.0
Previous gestational diabetes mellitus 3 hr, 100 gm oral	14 (17%)	3 (43%)	0.13
GTT (mg/dl)	96.5 ± 13.2	136.6 ± 24.8	0.0001
Fasting 1 hr	90.5 ± 13.2 206.5 ± 23.6	250.0 ± 24.8 250.4 ± 36.6	0.001
2 hr	185.0 ± 30.1	230.4 ± 30.0 224.3 ± 73.2	0.03
3 hr	128.2 ± 33.2	200.3 ± 68.2	0.005
Insulin therapy dur- ing gestation	32 (40%)	7 (100%)	0.003
Breast-feeding at time of postpartum oral GTT	35 (44%)	2 (29%)	0.69
Oral contraceptives at time of postpartum oral GTT	8 (10%)	0 (0%)	0.63

cluded women with impaired and nondiagnostic test results in our abnormal group because there is an increased risk for the development of type II diabetes mellitus in patients with impaired glucose tolerance,10 and the distinction between normal and impaired glucose tolerance (nondiagnostic category) is an arbitrary distinction on the far right end of the normal distribution curve of glucose values in nondiabetic subjects." Furthermore, when we analyzed the data from the seven patients with frank glucose intolerance in comparison with the patients with normal postpartum glucose tolerance, the most highly significant differences again were fasting glucose level, gestational age at the time of diagnosis of gestational diabetes, and the need for insulin therapy during gestation. We believe it is important to identify, as early as possible, all the women in these high-risk groups to institute long-term therapeutic recommendations for diet, activity, and achievement of ideal body weight.

The results of our study agree with those described by Kjos et al.⁷ They found a 19% (48/246) incidence of abnormal glucose tolerance in women with gestational diabetes in the early postpartum period. Furthermore, they also found that fasting glucose level and diagnosis of gestational diabetes before 24 weeks' gestation were additional risk factors for abnormal postpartum glucose tolerance.

Our study, however, differs from the previous study because of the different subject populations and definitions used to define glucose tolerance. Ninety-eight percent of the women examined by Kjos et al. in Los Angeles had Spanish surnames. Our subject population was predominantly white (82%), with lesser percentages of black and Hispanic subjects. There is a >15% rate of diabetes in Mexican-Americans 2 as compared with

the 5% to 7% prevalence of diabetes mellitus in whites.¹³ The difference in the prevalence of diabetes in the different study populations is potentially a confounding variable that needs to be considered when the results of the studies are compared.

The glucose concentrations that we used to define gestational diabetes were those of Carpenter and Coustan.⁸ In contrast, Kjos et al.⁷ used the criteria of the National Diabetes Data Group,⁹ which are approximately 6% higher than the glucose thresholds that we used. Hence, our subject population might be expected to include women with less severe glucose intolerance. Furthermore, we included women with nondiagnostic glucose tolerance in our abnormal group, whereas women with nondiagnostic postpartum glucose tolerance were classified as normal by Kjos et al.⁷ In spite of the differences in study populations and definition of glucose tolerance, both studies found that approximately 20% of women with gestational diabetes will have an abnormal postpartum glucose tolerance.

Forty-nine percent (51/103) of our study subjects required insulin therapy during gestation as compared with 15% to 20% in our overall gestational diabetic population. Hence, the patients most likely to complete postpartum glucose tolerance testing were those women who required more intensive therapy during pregnancy. Therefore the incidence of abnormal postpartum glucose tolerance may be lower if all women with gestational diabetes are tested.

As expected, there was a greater risk of an abnormal postpartum glucose tolerance in women with gestational diabetes who had many of the characteristics commonly associated with type II diabetes. They were significantly heavier and had a greater body mass index¹⁴ as compared with the women with normal post-

partum glucose tolerance. Furthermore, elevated fasting glucose level, gestational age at diagnosis, and insulin therapy were found to be the best predictors of abnormal postpartum glucose tolerance. These findings most probably represent either women with abnormal but unrecognized glucose intolerance before conception or women with more severe degrees of subclinical abnormalities of glucose metabolism made manifest by the stress of pregnancy.15 The pathophysiologic characteristics of these subclinical metabolic abnormalities (i.e., increased peripheral or hepatic insulin resistance, inadequate insulin response, or a combination of factors) that result in gestational diabetes or postpartum glucose intolerance have yet to definitively described. However, these results would further support early screening for gestational diabetes in women with multiple risk factors.

In summary, the results of this study support the recommendations of the Second International Workshop-Conference on Gestational Diabetes Mellitus⁶ that women with gestational diabetes have a postpartum oral GTT. Furthermore, women with elevated fasting glucose levels, diagnosis at an early gestational age, and possibly insulin therapy during pregnancy are at higher risk of having an abnormal GTT result. The early recognition and treatment of diabetes mellitus and abnormal postpartum glucose tolerance may help decrease the long-term morbidity and mortality in this high-risk population.

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Urinary protein/creatinine ratio before and during pregnancy in women with diabetes mellitus

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Quantitation of urinary protein excretion has traditionally involved collection of a 24-hour urine specimen. Recent reports have suggested that the ratio of protein to creatinine in a single-voided urine specimen may be used as a screening test of proteinuria, obviating the need for a 24-hour urine collection. This study was undertaken to determine whether the urinary protein/creatinine ratio was correlated with 24-hour protein excretion in women with diabetes, to determine whether pregnancy had any effect on the correlation, and to test the accuracy of estimates of 24-hour protein excretion on the basis of the protein/creatinine ratio. We studied 329 24-hour urine specimens from 133 women with classes B through RF diabetes. The protein/creatinine ratio was highly correlated with total protein excretion (r = 0.977, p < 0.0001). The correlation was not affected by pregnancy, trimester, or preeclampsia. Three methods were used to predict protein excretion on the basis of the ratio. Compared with actual protein excretion, predicted values had mean errors of 19% to 27%; 6% to 13% of predictions were in error by $\geq 50\%$. Because of these large errors, we conclude that this method of estimating protein excretion has limited value in pregnant women with diabetes. (AM J Obstet Gynecol. 1991;165:920-3.)

Key words: Proteinuria, diabetes mellitus, pregnancy

Urinary protein excretion is routinely assessed before and during pregnancy in women with diabetes to detect nephropathy and to follow its course. Quantitation of protein excretion is also of value in the diagnosis of preeclampsia. Standard diagnostic criteria for diabetic nephropathy and preeclampsia are based on 24-hour urinary protein excretion. However, 24-hour urine collections are inconvenient for patients, and incomplete collections are common.¹

Recent reports have shown that the ratio of protein to creatinine in a single-voided urine specimen has an excellent correlation with the 24-hour urinary protein excretion, suggesting that this ratio might be a useful screening test of protein excretion. The correlation has been validated in healthy nonpregnant subjects, and renal disease, and pregnant women with and without pre-eclampsia. It has not been validated in pregnant women with diabetes.

This study was undertaken to determine whether the urinary protein/creatinine ratio was correlated with 24-

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Reprint requests: C. Andrew Combs, MD, PhD, Division of Maternal-Fetal Medicine (ML-526), University of Cincinnati College of Medicine, Cincinnati, OH 45267-0526. 6/6/30896 hour protein excretion in women with diabetes, to determine whether pregnancy had any affect on the correlation, and to test the accuracy of several methods of estimating 24-hour protein excretion on the basis the protein/creatinine ratio.

Methods

Records were reviewed from all women followed in the Diabetes and Pregnancy Program at Children's Hospital of San Francisco and Moffitt Hospital, University of California, San Francisco. Inclusion criteria were diagnosis of diabetes mellitus before pregnancy, enrollment before 16 weeks of gestation, and delivery from 1982 through 1989. Women with gestational diabetes were not included.

The protocol for management of diabetes in pregnancy included a 24-hour urine collection for measurement of total protein and creatinine clearance before pregnancy (if possible) and at or near the end of each trimester of pregnancy. Total protein was measured by the benzethonium chloride method⁷ or the Coomassie brilliant blue method⁸ and expressed in grams per day. Urinary creatinine (grams per day) was measured by the Jaffe reaction. Specimens were considered inadequate and were excluded from analysis if total creatinine excretion was <10 mg/kg/day or if there was evidence of bacteriuria. Protein/creatinine ratio (a unitless quantity) was calculated from the total protein and total creatinine values of each 24-hour specimen.

For the initial analyses, specimens were grouped ac-

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Table I. Correlation between protein/creatinine ratio and 24-hour protein excretion

Trimester	No.	Regression line	r	R^2	p Value
Prepregnant	35	$TP = 1.26 \times PCR - 0.03$	0.975	0.951	< 0.0001
First	109	$TP = 1.10 \times PCR + 0.03$	0.971	0.942	< 0.0001
Second	106	$TP = 1.12 \times PCR + 0.03$	0.995	0.989	< 0.0001
Third	79	$TP = 1.22 \times PCR - 0.02$	0.966	0.934	< 0.0001
Pooled data	329	$TP = 1.15 \times PCR + 0.02$	0.977	0.955	< 0.0001

r, Pearson product-moment correlation coefficient; TP, total protein excretion (grams per day), PCR, protein (grams)/creatinine (grams) ratio.

cording to trimester of collection. Within each group, the linear correlation between protein/creatinine ratio and 24-hour total urinary protein was calculated with least-squares regression. Between-group differences in the regression lines were tested with analysis of covariance, and between-group differences in total creatinine excretion were tested with repeated-measures analysis of variance. Because these analyses showed no significant differences, subsequent analyses were performed after the data from prepregnancy and all three trimesters were pooled.

Three methods for estimating 24-hour urinary protein excretion on the basis of the protein/creatinine ratio were evaluated. The first two methods were based on the formula:

Predicted
$$TP = PCR \times CREAT$$

where TP is 24-hour total protein excretion (grams), PCR is the protein/creatinine ratio (grams per gram, a unitless quantity), and CREAT is 24-hour total creatinine excretion (grams). In the first method total creatinine excretion was calculated from the formula of Cockroft and Gault10:

$$CREAT = [(140 - Age) \times (Weight) \times 0.85]/5000$$

where creatinine excretion is in grams per day, age is in years, and weight is in kilograms. In the second method 24-hour total creatinine excretion was taken as the observed mean value of 24-hour creatinine excretion (1.26 gm/day). The third method was based on the formula:

Predicted
$$TP = (PCR \times Slope) + Intercept$$
,

where slope is the slope of the pooled regression line relating the 24-hour total protein excretion to the protein/creatinine ratio (1.15 gm), and intercept is the y-intercept of that line (0.02 gm). For all three methods, the predicted value of the 24-hour total protein excretion was compared with its actual value using leastsquares regression and by calculating the mean percentage error and the fraction of predicted values of the 24-hour total protein excretion that differed from the actual value by >50%.

All statistical analyses were performed with SPSS.11 All p values < 0.05 were considered significant. Values are expressed as mean ± 1 SD.

Table II. Total creatinine excretion by trimester

Trimester	No.	Creatinine excretion (gm/day)		
Prepregnant	35	1.20 ± 0.24		
First	109	1.30 ± 0.28		
Second	106	1.27 ± 0.30		
Third	79	1.22 ± 0.31		
Pooled data	329	1.26 ± 0.29		

Data are mean \pm 1 SD.

Results

One hundred fifty-eight consecutive women met the inclusion criteria. Twenty-five women were excluded because they had no adequate urine collections. In the remaining 133 women there were 329 adequate specimens; 35 of these women had adequate collections before pregnancy.

Maternal age averaged 30.9 ± 5.0 years, and the average interval from diagnosis of diabetes was 12.5 ± 7.6 years. Fifty women were in White class B, 36 in class C, 18 in class D, 20 in class F, 6 in class R, and 3 in class RF. By the time of the third-trimester collection, 17 had preeclampsia, defined as a sustained increase in blood pressure > 140/90 mm Hg with proteinuria ≥ 300 mg/day.

Urinary protein/creatinine ratio was linearly correlated with 24-hour protein excretion before pregnancy and in all three trimesters. The regression statistics are summarized in Table I. Analysis of covariance showed no differences in the slopes or intercepts of the regression lines between the four groups, so the results were pooled to derive a final regression line. The pooled data are plotted in Fig. 1. The two panels show that the strong linear relation applied for high values of urinary total protein, as well as for low values. The presence of preeclampsia had no significant effect on the regression.

Table II shows the values of total creatinine excretion from before pregnancy and in all three trimesters. Analysis of variance showed no significant difference between the groups.

Table III compares the performance of the three methods for estimating 24-hour total protein excretion on the basis of the protein/creatinine ratio. The values of total protein predicted with the Cockroft-Gault for-

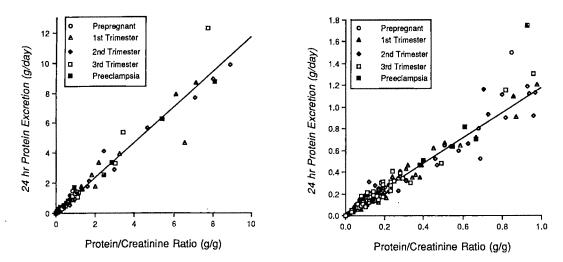


Fig. 1. Relation of total protein excretion to protein/creatinine ratio before pregnancy (circles), in first trimester (triangles), in second trimester (diamonds), and in third trimester with (hatched squares) and without (open squares) preeclampsia. Left panel, All subjects. Right panel, Detail of subjects with protein/creatinine ratio < 1. Regression line for pooled data: y = 1.15 x + 0.02.

Table III. Accuracy of prediction of 24-hour protein excretion from protein/creatinine ratio

	,	Regression of precon actual T					
Method	Formula	Slope	Intercept	r	Mean error	Errors >50%	
Cockroft-Gault	$TP = PCR \times (140 - Age) (Wt) \times 0.85$ 5000	1.32	0.05	0.982	27% ± 31%	13% ·	
Mean creatinine Regression	$TP = PCR \times 1.26$ $TP = PCR \times 1.15 + 0.02$	1.09 1.00	$0.02 \\ 0.00$	$0.977 \\ 0.977$	$19\% \pm 20\%$ $24\% \pm 29\%$	6% 9%	

TP, Total protein (grams per day); r, Pearson product-moment correlation coefficient; PCR, protein (grams)/creatinine (grams) ratio; Age, maternal age (years); Wl, maternal weight (kilograms).

mula were strongy correlated with the actual values (r=0.981), but the slope of the regression line was 1.32, indicating that the formula overestimated protein excretion. As a result, predicted values differed from actual values by $27\% \pm 31\%$; indeed, 13% of the predicted values differed from the measured values by >50%. These errors occurred, in part, because measured total creatinine excretion was not significantly correlated with the creatinine excretion predicted by the Cockroft-Gault formula (r=0.097; p>0.05). Indeed, measured creatinine excretion was only weakly correlated with maternal age (r=0.129, p<0.02) or body weight (r=0.129, p<0.02).

The other formulas resulted in slightly smaller average errors between actual and predicted values, but both formulas resulted in a considerable fraction of patients in whom there was an error of >50% between measured and predicted values.

Comment

Because the method can be applied to a single-voided urine specimen, quantitation of urinary protein from

the protein/creatinine ratio has potential advantages over collection of a 24-hour specimen. In a prospective study, 35% of women who were asked to complete a 24-hour collection failed to provide an adequate specimen, either because of noncompliance or because of inadequate urine volume. The protein/creatinine ratio, in principle, is not dependent on urinary volume and can be applied to any random urine specimen. Further, the method does not impose a mandatory 24-hour delay to obtain a specimen, so that results can be immediately available. This may be useful in the management of preeclampsia, where rapid increases in urinary protein excretion may indicate a need to expedite delivery.

There are also some major disadvantages to the estimation of protein excretion on the basis of the protein/creatinine ratio. First, the standard diagnostic criteria for both diabetic nephropathy and preeclampsia are based on 24-hour protein excretion.^{12, 13} Because there are no accepted diagnostic standards that are based on protein/creatinine ratio, it is necessary to calculate a daily protein excretion from the ratio. Algebraically, this is accomplished by multiplying the ratio

by the 24-hour total creatinine excretion. However, unless a 24-hour specimen is collected for measurement of creatinine excretion, it is necessary to assume some value of creatinine excretion. In this study the predicted values of protein excretion that are based on the protein/creatinine ratio were highly correlated with the actual values (Table III), but all three methods yielded a considerable percentage with large errors. Thus values of daily protein excretion derived from protein/creatinine ratios must be regarded only as estimates.

Another disadvantage, not addressed by these data, is that both protein excretion and creatinine excretion have diurnal variations.14, 15 Because protein excretion is elevated by dietary protein and exercise, a urine specimen obtained late in the day may have a higher protein concentration than an overnight specimen. While there may be less day-to-day variability in first-morning voided specimens than in random specimens or 24hour specimens,5 first-morning specimens have poor sensitivity and specificity in the diagnosis of diabetic nephropathy and incipient nephropathy.15 Further, in preeclampsia protein excretion rates can vary from hour to hour,16 and thus a single-voided urine specimen may not accurately reflect average protein excretion.

Given these disadvantages, we believe that estimation of urinary protein excretion on the basis of the protein/creatinine ratio has limited value in pregnant women with diabetes. Two potential roles for this method are in patients who are noncompliant with 24hour urine collections or in patients with preeclampsia in whom the information is needed rapidly. However, even in these patients, it must be recognized that large errors in estimation can occur and it is not clear that the method is better than qualitative dipstick testing.

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Drug abuse screening of childbearing-age women in Alabama public health clinics

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During August 1989, the urine of 5010 women of childbearing-age in Alabama was screened for marijuana, cocaine, opiates, amphetamines, and barbiturates. The data base consisted of 2970 pregnant women, 374 of whom were at high risk, and 2019 nonpregnant women. Our findings included the following information: (1) The statewide prevalence of positive screens for any drugs tested was 12.9% for all women (11.0% for those who were pregnant and 15.6% for those who were not pregnant). (2) There was no difference between urban and rural groups for any drugs tested. (3) Positive marijuana screens were increased among white and nonpregnant women (p < 0.01). (4) Positive cocaine screens were increased among black and single women (p < 0.01). (5) More screens were positive in women older than age 20 (p < 0.01). (6) There was no difference between pregnant and nonpregnant women for positive cocaine screens. (7) No difference existed among the trimesters of pregnancy for positive cocaine screens. (8) Positive screens for marijuana were more frequent in the first trimester of pregnancy than in the second trimester (p = 0.02) or the third trimester (p = 0.001). (9) There was no difference between high-risk and low-risk maternity patients for any drugs tested. (AM J OBSTET GYNECOL 1991;165:924-7.)

Key words: Drug abuse, prevalence

More than 5 million American women of childbearing age use illicit drugs. Almost 1 million of these women use cocaine, and 3.8 million use marijuana.¹ Numerous studies have examined known drug users or have assessed the effects of illicit drug use among pregnant women and their infants.²-⁴ However, we are not aware of any previous assessments of the statewide prevalence of drug use in pregnant and nonpregnant women of childbearing age.

This study was designed to determine the prevalence of illicit drug use among women enrolled in maternity and family planning clinics in Alabama and among women enrolled in a high-risk obstetric referral clinic.

Background

Alabama is classified as an urban state with scattered rural areas.⁵ Public health maternity services are available in all counties for pregnant women with incomes

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This study was conducted in Alabama public health clinics.

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Reprint requests: Sherry K. George, MPA, Alabama Department of Public Health, 434 Monroe St., Montgomery, AL 36130-1701. 6/6/30773 below 150% of the federal poverty level. Maternity patients are screened for risk factors at each visit. Family planning services are available to anyone but were developed for women with incomes below 150% of the federal poverty level.

Most patients referred to the Division of Maternal-Fetal Medicine Obstetric Complications Clinic (OBCC) of the University of Alabama at Birmingham are from public health maternity clinics. In the OBCC patients are grouped into three areas: high-risk or intermediaterisk obstetric complications, prematurity prevention, or hypertension prevention.

Methods

Our study population consisted of pregnant and nonpregnant women enrolled in public health maternity and family planning clinics or in the OBCC at the University of Alabama at Birmingham. A nonexperimental descriptive research design was used. Urine samples from anonymous patients were tested for marijuana, cocaine metabolites, opiates, amphetamines, and barbiturates. Subject consent was not obtained. It was assumed that positive findings for any of the drugs indicated illicit use.

Urine samples and corresponding demographic data were collected from Aug. 21, 1989, to Sept. 1, 1989. Urine samples obtained for routine testing procedures at each patient visit were used. At the usual point of discard, excess urine from each sample was poured or injected into a plain red Vacutainer blood collection tube. The tubes were one-half to three-quarters filled,

Table I. Patient characteristics and prevalence of drug use detected among selected population of women of childbearing age in Alabama, 1989

	, Scree	med .		Type of drug					
Characteristic	No.	·%	Any drug (%)	Marijuana (%)	Cocaine (%)	Barbiturates (%)	Amphetamines (%)	Opiates (%)	
Race	,						*	!	
White	2393	47.8	17.3	15.8	0.9	0.6	0.5	0.5	
Black	2553	50.9	8.8	7.1	1.9	0.6	0.1	0.3	
Age (yr)						****	011	0.0	
<20	1727	34.5	8.0	6.9	0.3	0.5	0.3	0.3	
>20	3240	64.7	15.3	13.4	1.9	0.7	0.3	0.5	
Marital status						• • • • • • • • • • • • • • • • • • • •		0.0	
Married	1753	35.0	13.7	12.2	0.7	0.7	0.3	0.6	
Single	3180	63.5	12.2	10.6	1.8	0.6	0.2	0.3	
Parity									
0 '	1870	37.3	10.5	8.7	1.2	0.7	0.2	0.3	
1-3	2373	47.4	14.2	12.4	1.6	0.6	0.3	0.4	
>3	765	15.3	15.3	14.0	1.2	0.5	0.4	0.5	
Population									
Úrban	3600	71.9	12.8	11.2	1.5	0.5	0.2	0.4	
Rural	1410	28.1	13.1	11.5	1.1	0.9	0.4	0.5	
Patient category						1			
Nonpregnant	2019	40.3	15.6	14.1	1.4	0.5	. 0.4	0.7	
Pregnant	2970	59.3	11.0	9.3	1.4	0.7	0.2	0.2	
OBČC*	(374)		12.0	9.4	. 1.1	1.6	0.3	0.3	
Gestational age (wk)						•		•	
0-13	378	12.7	15.6	14.8	0.8	0.5	0.0	0.3	
14-27	927	31.2	11.8	10.3	1.6	0.7	0.2	0.1	
28-delivery	1366	46. 0	9.4	7.5	0.8	1.0	0.2	0.3	
TOTAL	5010	100.0	12.9	11.3	1.4	0.6	0.3	0.4	

Numbers and percents may not add to total as a result of unknown factors.

and the stoppers were secured with tape if the injection method was not used. No identifying information was placed on the tubes. As the samples were obtained, each patient's chart was marked with an identifier (sticker). Thus no duplicate samples were submitted. Demographic data were obtained from a review of the patients' charts, but no identifying information was included. All urine samples and demographic data forms were sent to the state laboratory in Montgomery, Alabama.

Drug screening for amphetamine or methamphetamine, barbiturates, cannabinoids, cocaine metabolites, and opiates was performed by means of a fluorescence polarization immunoassay. All positive samples underwent a second analysis. The manufacturer's recommended cutoff levels for determining the presence of drugs and drug metabolites were used to report the results. The manufacturer claims 99% to 100% agreement with gas chromatography—mass spectrometry for positive specimens and 95% to 100% agreement for negative specimens. Because many over-the-counter drugs produce metabolites that mimic amphetamine and methamphetamine, positive specimens in this category were re-tested with thin-layer chromatography. Only the amphetamine-confirmed specimens were in-

cluded as positives. No further confirmatory testing was performed on positive specimens in the other drug categories. We specifically wished to evaluate the prevalence of illicit drug use among rural and urban populations, among white and black populations, among single and married women, in relation to age, among pregnant and nonpregnant women, in relation to parity and current pregnancy, in relation to the trimesters of pregnancy, in a high-risk referral obstetric clinic population compared with a low-risk referral obstetric clinic population (women enrolled in research-oriented clinical trials), among women referred to a high-risk obstetric clinic because of a history of substance abuse who were compared with those referred for other complications, and among high-risk obstetric referral patients compared with the low-risk public health maternity population from the same urban county.

After laboratory analysis, all patient demographic data were entered into a computer by means of Professional File (Software Publishing Corp., Mountain View, Calif.). All computations (percentages, measures of central tendency [means], and measures of dispersion [standard deviations]) were computed with Lotus. Data results were computed with χ^2 analysis and Fisher's exact test.

^{*}Subgroup of pregnant population.

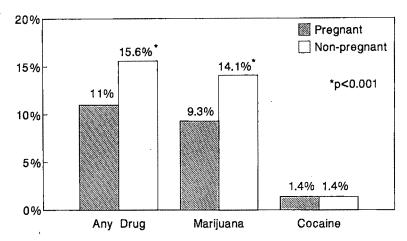


Fig. 1. Statewide prevalence of illicit drug use by pregnancy status.

Results

Statewide population. Six hundred forty-six patients (12.9%) tested positive for at least one of the drugs (Table I). Specifically, 11.3% tested positive for marijuana, 1.4% for cocaine, 0.6% for barbiturates, 0.4% for opiates, and 0.3% for amphetamines.

In the 646 women with positive screens, the drug most frequently used was marijuana (87.5%), which was followed by cocaine (10.7%), barbiturates (4.8%), opiates (3.1%), and amphetamines (2.0%). Fifty-two women (12.4%) tested positive for two or more of the drugs. Of the women who had multiple positive screens, 41 (78.8%) tested positive for cocaine and marijuana.

Various demographic characteristics of the group that had positive drug screens are presented in Table I. Positive screens for any drug and for marijuana were higher in white women, who were two times more likely to test positive for any drug or for marijuana than were black women (p < 0.001). Black women were 2.1 times more likely to test positive for cocaine than were white women (p < 0.003). The use of any drug or marijuana or cocaine was more frequent in women older than age 20 than in teenagers (p < 0.001). The mean age of women who tested positive for any drug was 23.8 years (SD, +4.93).

Single women were 2.6 times more likely to test positive for cocaine than were married women (p < 0.002). There was no difference in the marital status of women who tested positive for any drug or for marijuana. Multiparous women had significantly more positive screens for any drug and for marijuana than did nulliparous or primigravid women (p < 0.001); however, there was no significant difference in positive cocaine screens among these groups. The urban-rural distribution was similar among women who tested positive for any of the drugs.

Of the 2970 pregnant women screened, 326 (11.0%) tested positive for at least one of the drugs, as compared with 315 (15.6%) of the 2019 nonpregnant women

screened. Nonpregnant women were 1.5 times more likely to have a positive screen for any drug or for marijuana than were pregnant women (p < 0.001). Pregnant and nonpregnant women had a similar prevalence of positive cocaine screens (Fig. 1). Among statewide pregnant women, positive screens for marijuana were more frequent in the first trimester of pregnancy than in the second trimester (p = 0.02) or the third trimester (p = 0.001). The frequency of positive cocaine screens was similar in each trimester. Overall, the highest percentages of positive cocaine screens occurred in the urban areas of Mobile and Birmingham (2.2% and 2.1%, respectively).

Obstetric referral high-risk population. Forty-five (12%) of the 374 OBCC patients screened tested positive for at least one of the drugs. Forty (14.2%) of the 282 women enrolled in the high-risk or intermediaterisk obstetric complications clinics or the prematurity prevention clinic had a positive urine screen. A positive screen was seen in only 7 (7.6%) of the 92 women enrolled in the low-risk Hypertension Prevention Project (p = 0.098, NS).

Fifteen (4%) of the 374 OBCC patients tested were referred because of a history of alcohol and/or drug abuse. Eleven of these 15 (73%) had a positive drug screen; two of these 11 tested positive for more than one drug. Conversely, 26 (9.6%) of the women with a complication of pregnancy other than drug abuse (n = 267) had a positive urine screen (p < 0.001).

A total of 1104 maternity patients were screened in urban Jefferson County–Birmingham. In this county women enrolled in the OBCC had a frequency of positive urine screens (30/220) similar to that found among women considered to be at low-risk (88/884; p=0.11, NS).

Commen

Drug use is known to be common among pregnant women in the United States. The Rhode Island Department of Public Health found that 7.5% of women

in labor tested positive for at least one illicit drug.7 In Pinellas County, Florida,8 it was found that nearly 15% of the pregnant women studied tested positive for alcohol and drug use (12.0% tested positive for marijuana and 3.4% were positive for cocaine).

Our report provides a view of illicit drug use among pregnant and nonpregnant women aged 15 to 44 years in a statewide public health population. The prevalence of overall illicit drug use and marijuana use was higher in white women than in black women. However, black women were almost twice as likely to test positive for cocaine. The increased use of cocaine in black pregnant women is similar to that reported from Rhode Island and Florida. We found no difference between urban and rural populations for any of the drugs tested. Chasnoff⁹ found that the prevalence of drug use (particularly cocaine) in pregnancy was similar in different geographic areas of the country, regardless of the hospital or the size of the population studied.

In a congressional hearing, Ja Joon testified that "cocaine-using mothers typically are not first-time, adolescent mothers, but older women, who do not plan for their pregnancies."10 We confirmed a significantly higher prevalence of cocaine use (as well as use of any drug or marijuana) among women >20 years old. We also found that the use of any drug was significantly higher among women who had one or more children.

Our pregnant and nonpregnant populations were similar with respect to the frequency of a positive urine screen for cocaine (1.4%). Of the drugs targeted in our study, the potential adverse effects of cocaine use during pregnancy are best known. However, the potential for pregnancy complications and/or poor perinatal outcomes is increased when any of these drugs is abused.11-15 Thus we surmised that women identified as having high-risk pregnancies may have a higher prevalence of illicit drug use than do women who are considered to have low-risk pregnancies. Our results did not support this hypothesis. A higher prevalence of illicit drug use was found only among those who were referred to the OBCC because of a known drug abuse

Our report was limited to randomly selected women from the public health sector who were seen during a specific 2-week period. Women from the private sector were not screened. The assignment of urban or rural status was based solely on population density. Per capita income and economic activity were not considered. Urine screening has a short half-life. Thus, because serial testing was not performed, it is possible that the total drug exposure of fetuses may have been substantially underestimated in this population.

This article is dedicated to the memory of Sue Jones, who so graciously spent many hours computing the data for this project. Other individuals acknowledged for their contributions to this paper are Dr. Bruce Harris and Dr. Charles Woernle, editorial review; Fern Shinbaum, Lee Rawlinson, and Jim Laney, study development; and Sue Cliver, statistical analysis.

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Shoulder dystocia and birth trauma in gestational diabetes: A five-year experience

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Over a 5-year period, 210 patients with gestational diabetes mellitus were delivered of offspring weighing ≥3500 gm. Only three primary cesarean sections were performed electively because of suspected macrosomia. One hundred twenty patients were delivered vaginally. There were 15 shoulder dystocias but only one permanent brachial plexus injury. Seven of the 15 shoulder dystocias occurred in offspring weighing <4000 gm. Of variables examined, only the use of forceps was clearly associated with an increased risk of shoulder dystocia (odds ratio, 5.1). A policy to deliver by cesarean section all fetuses estimated to weigh >4000 gm would considerably increase the number of cesarean sections with minimal fetal benefit. (AM J OBSTET GYNECOL 1991;165:928-30.)

Key words: Shoulder dystocia, birth trauma, gestational diabetes

Shoulder dystocia is a potentially serious complication in pregnancies complicated by diabetes mellitus¹ and has been noted in 13% to 31% of pregnancies with an infant weighing ≥4000 gm.². ³ These observations have led some to recommend elective cesarean section for diabetic patients when the estimated fetal weight is >4000 gm.².⁴ Inherent problems with this scheme are the inaccuracies in estimation of fetal weight and the maternal morbidity associated with cesarean section.⁵ Additionally, it is uncertain whether the risk of shoulder dystocia is as high in gestational diabetes mellitus as it is in pregestational insulin-dependent diabetes mellitus, and most studies have used a heterogeneous population of both to generate clinical recommendations.².⁴

Because of these issues we have adopted a liberal philosophy in patients with gestational diabetes mellitus, encouraging an attempt at vaginal delivery as long as labor progresses normally and discouraging primary cesarean sections for the sole indication of an estimated fetal weight >4000 gm. This philosophy permitted us to examine the frequency of shoulder dystocia and birth trauma in a large population of patients with gestational diabetes mellitus allowed a trial of labor.

Material and methods

Between Oct. 1, 1983, and July 30, 1989, 210 patients with gestational diabetes mellitus whose neonates

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Reprint requests: James D. Keller, MD, 1875 Dempster, Suite 325, Park Ridge, IL 60068. 6/6/30771 weighed ≥3500 gm were identified by means of the Prentice Women's Hospital perinatal data base. During this time period, women obtaining prenatal care underwent universal screening for gestational diabetes mellitus. The diagnosis was established with the O'Sullivan-Mahan criteria modified for plasma glucose.6 Patients who remained normoglycemic on diet therapy were denoted A₁ gestational diabetes mellitus. If fasting plasma glucose level exceeded 105 mg/dl or the postprandial plasma glucose was elevated (1 hour, >140 mg/dl), the patient was denoted A2 gestational diabetes mellitus, and insulin therapy was initiated unless the patient was ≥36 weeks' gestation. Patients with a fasting plasma glucose level ≥130 mg/dl were excluded from this study because of the likelihood that these patients had unrecognized pregestational diabetes.

The following variables were examined: severity of maternal carbohydrate intolerance, parity, use of oxytocin for augmentation of labor, length of second stage of labor, mode of delivery, birth weight, occurrence of shoulder dystocia, and presence or absence of birth injury. Shoulder dystocia was diagnosed by the physician attending the delivery. Follow-up of offspring discharged from the hospital with a brachial plexus injury was obtained from either the parent or pediatrician of the injured neonate.

Statistical analysis was performed by Student t test for means of continuous variables. Odds ratios were calculated for discrete data with 95% confidence intervals.

Results

Of the 210 mother-infant pairs, 173 underwent a trial of labor. Three patients were delivered by primary cesarean section because of suspected macrosomia before

Table I. Mode of delivery after trial of labor

	Birth weight				
	3500-3999 gm	4000-4499 gm	>4500 gm		
No.	105	56	12		
Cesarean section	30 (29%)	19 (34%)	4 (33%)		
Vaginal delivery	75 (71%)	37 (66%)	8 (67%)		
Spontaneous	71 `	33	8		
Forceps	4	4	0		

Table II. Incidence of shoulder dystocia and birth trauma in patients delivered vaginally

	Birth weight				
	3500-3999 gm	4000-4499 gm	>4500 gm		
No.	75	37	8		
Shoulder dystocia	7 (9%)	5 (14%)	3 (38%)		
With clavicular fracture	2 ` ′	0	0 `		
With brachial palsy	2	I	1		
With residual palsy	. 0	1	0		

Table III. Frequency of shoulder dystocia by risk factor

	No.	Shoulder dystocia	· Odds ratio	95% Confidence interval
A ₁ gestational diabetes mellitus	79	9 (11.4%)	0.78	0.25-2.27
A ₂ gestational diabetes mellitus	41	6 (14.6%)		
Second stage of labor				
Normal	104	11 (10.6%)	2.36	0.55 - 7.98
Prolonged	16	4 (25%)		
Oxytocin augmentation				
No	98	13 (13.3%)	0.69	0.08-3.0
Yes	22	2 (9.1%)		
Parity		,		
Multiparous	90	9 (10%)	0.50	0.16-1.7
Nulliparous	30	6 (20%)		•
Delivery		• • •		
Spontaneous	112	11 (9.8%)	5.1	1.2-17.2
Forceps	8	4 (50%)		

onset of labor (actual birth weight, 3800, 3880, and 4460 gm); both infants weighing <4000 gm were estimated by ultrasonography to be >4000 gm (4388 and 4436 gm, respectively). Thirty-four patients had elective repeat cesarean sections. The mode of delivery for the remaining 173 patients is shown in Table I. (Eight of 13 attempts at vaginal birth after cesarean section were successful with no occurrences of shoulder dystocia.)

The incidence of shoulder dystocia and birth trauma in the 120 patients who ultimately were delivered vaginally is illustrated in Table II. Of the four brachial plexus injuries, two resolved before discharge from the hospital, and one resolved by I month of age. One infant (birth weight, 4220 gm) continues to show mild residual impairment 4 years after delivery.

The birth weight was greater, but not significantly

different, in deliveries complicated by shoulder dystocia (4094 ± 517 vs 3933 ± 317 gm, respectively; p = 0.08). As can be seen in Table III, in those patients delivered vaginally neither A_2 gestational diabetes mellitus, use of oxytocin for augmentation of labor, nor nulliparity increased the risk of shoulder dystocia. Use of forceps, however, did increase the risk of shoulder dystocia (birth weights, 3700, 4100, and 4135 gm) approximately fivefold. Prolonged second stage of labor (>60 minutes in multiparous women, >120 minutes in primiparous women) was associated with a trend toward an increased risk of shoulder dystocia (odds ratio, 2.36; 95% confidence interval, 0.55 to 7.98).

Comment

In spite of continued improvement in perinatal outcome in gestational diabetes, shoulder dystocia and sub-

sequent birth trauma remain serious complications. It is known that offspring of diabetic mothers are at greater risk of shoulder dystocia than are the offspring with similar birth weights of normoglycemic women. This is believed to be due to proliferation of insulinsensitive tissue that causes a relative disproportion in the size of the fetal chest and shoulders relative to the fetal head.7 Acker et al.,2 in a restrospective review of shoulder dystocia, reported a 31% incidence in deliveries involving maternal diabetes and a birth weight >400 gm. No distinction was made between those with gestational diabetes mellitus versus insulin-dependent diabetes mellitus. In our series shoulder dystocia occurred less frequently (8 of 45 vaginal deliveries with birth weight ≥4000 gm). This may reflect differing clinical criteria for the diagnosis of shoulder dystocia, a problem inherent in comparing retrospective studies. Alternatively, there may be a lower incidence of shoulder dystocia in gestational compared with insulin-dependent diabetes mellitus.

Our study failed to show that more severe carbohydrate intolerance (A₂ gestational diabetes mellitus), use of oxytocin, or nulliparity increased the risk of shoulder dystocia. There was a trend toward an increased risk of shoulder dystocia when the second stage of labor was prolonged, but the only significant increase occurred in vaginal deliveries accomplished by forceps. Replacing forceps delivery with cesarean section would have reduced shoulder dystocia by >25%. On the basis of our findings, forceps should be used with caution in pregnancies complicated by gestational diabetes mellitus when the birth of a large infant is anticipated.

Several strategies have been suggested to reduce the risk of shoulder dystocia in diabetes, including the liberal use of cesarean section.^{2,4} If perfectly executed, a

protocol to deliver every fetus >4000 gm by cesarean section would have increased the number of nonelective cesarean sections from 53 to 98 in our series. This excess of 45 cesarean deliveries would have obviated eight of 15 shoulder dystocias and one permanent brachial plexus injury. Although one could argue that such an approach is justified on the basis of the serious nature of the pediatric injury, it would not be possible to perfectly execute such a protocol in that there are serious limitations to the accurate estimation of fetal weight. Furthermore, almost half the instances of shoulder dystocia and birth trauma occurred in offspring weighing <4000 gm. Consequently, we continue to believe that to deliver by cesarean section all fetuses estimated to weigh >4000 gm would considerably increase the number of cesarean sections performed but not eliminate the risk of shoulder dystocia.

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Administration of heparin by subcutaneous infusion with a programmable pump

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Heparin has a short half-life (8 to 12 hours) and therefore must be administered by continuous infusion or by intermittent subcutaneous injection. Intermittent subcutaneous injection may lead to fluctuation in the levels of anticoagulation attained. In correcting this deficiency, the programmable automated subcutaneous infusion pump in conjunction with weekly home nursing visits has been used. Eight pregnant women with documented deep venous thrombosis or embolic events before pregnancy who received such therapy were studied. Eight similar subjects who received intermittent subcutaneous injection, matched for age, parity, site of deep venous thrombosis, and days on a regimen of heparin therapy, served as the control group. The mean daily dose of heparin by subcutaneous infusion pump was higher (29,445 vs 13.822 U). resulting in smoother, more therapeutic heparinization (mean partial thromboplastin time, 20.6 vs 10.4 seconds above control) when compared with the intermittent subcutaneous injection group (p < 0.05, $\rho < 0.007$). There were two complications (hematoma, site infection) in the intermittent subcutaneous injection group while none occurred in the subcutaneous infusion pump group. When used in concert with weekly home visits, the subcutaneous infusion pump method of administration allowed more even control of anticoagulation, appeared to result in fewer complications (although not statistically significant), and subjectively was better received by patients than the intermittent subcutaneous injection technique. (Am J OBSTET GYNECOL 1991;165:931-3.)

Key words: Deep venous thrombosis, anticoagulation, programmable external infusion pump

Deep venous thrombosis with subsequent pulmonary embolism is diagnosed with increased frequency during pregnancy.^{1,2} The incidence of deep venous thrombosis complicating gestation ranges from 0.05% to 1.8% and is most common after cesarean birth (2.2% to 3%). Its occurrence after vaginal birth is less frequent (0.5% to 1.2%). History of a thromboembolic complication, particularly if it occurred during a prior gestation, is considered to increase the risk of such problems in the current pregnancy to approximately 12%.4 Pulmonary embolism occurs in up to 16% of patients with untreated deep venous thrombosis, yielding a mortality rate of approximately 15%.1, 2, 5 Anticoagulation, if the drug is appropriately administered, will reduce the risk of pulmonary embolism and subsequent risk of mortality to 0.7%.4

Anticoagulation therapy in ambulatory patients usually employs warfarin, a drug contraindicated during

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gestation because of its teratogenic effects. Therefore parenteral administration of sodium heparin has been used on an outpatient basis as treatment for the pregnant patient. Heparin use is not without risk; hematomas, hemorrhage, hypersensitivity, alopecia, osteoporosis, and thrombocytopenia have been reported. 7-10

Heparin is usually administered in ambulatory patients by intermittent subcutaneous injection that involves two to four injections daily of 5000 to 10,000 U each. This therapy involves multiple injections that the patient may find objectionable, leading to diminished compliance. This method of administration also is associated with fluctuation of the level of anticoagulation. To address patient compliance and comfort many physicians reduce the number of injections per day, a practice that may result in inadequate anticoagulation.

In an effort to cope with these problems, it has been suggested that heparin be administered by continuous subcutaneous infusion with a programmable pump. ¹¹ Theoretically, this would allow for a more consistent, therapeutic level of anticoagulation while obviating the patient's need to perform multiple self-injections.

The purpose of the current study was to evaluate the use of the new automated programmable external infusion pump (model 404-SP, MiniMed Technologies,

Table I. Patient characteristics

	No.	Age (yr, mean ± SD)	Race*	Parity	Diagnosis
Programmable pump	8	29.9 ± 3.7	2/6	3.1 ± 1.4	Deep venous thrombosis $(n = 5)$ Pulmonary embolism
Intermittent subcutaneous injection	8	28.6 ± 4.7	2/6	3.3 ± 1.5	(n = 3) Deep venous thrombosis (n = 6) Pulmonary embolism (n = 2)

^{*}White/nonwhite.

Table II. Outcome statistics

	Programmable pump	Subcutaneous injection	p Value
Gestational age at onset of therapy (wk)	27 ± 4.4	26.4 ± 6.4	NS
Days of therapy	82.6 ± 32	83.6 ± 49.7	NS -
Amount of heparin per day (U)	$29,445 \pm 8572$	$13,822.5 \pm 4461$	p < 0.05
Mean partial thromboplastin time from control (sec)	20.6 ± 7.1	10.4 ± 6.0	p < 0.007
Complications	0 .	2	NS

Sylmar, Calif.) in an effort to achieve adequate anticoagulation in patients with thromboembolic disease.

Material and methods

A retrospective, paired, case-controlled study of 16 women was undertaken to evaluate the efficacy and complications from the automated external infusion pump for heparin infusion during pregnancy. Over a 9-month period, 8 patients were treated with the automated external infusion pump with comparison of their results to those of 8 women who were managed with intermittent subcutaneous injections of heparin during pregnancy. The patients were matched for age, parity, race, risk factor, and gestational age at onset of therapy. Patients managed with self-administered, intermittent, subcutaneous injections of heparin were treated every 8 to 12 hours utilizing standard dosing techniques.1 Patients who self-administered heparin were seen once per week in the physician's office to monitor coagulation parameters and assess the progress of the pregnancy.

The MiniMed model 404-SP external infusion pump with the MiniMed Soft Set (registered trademark of MiniMed Technologies) subcutaneous infusion set was used to administer the subcutaneous infusion of heparin in the treatment group. These patients were monitored by a perinatal nurse (Tokos Medical Corp., Santa Ana, Calif.) twice weekly with home visits for the first 2 weeks of the infusion. They received weekly home visits and prenatal visits to the physician each week for the remainder of the pregnancy. Perinatal nurses were available by telephone 24 hours per day. The patients in the subcutaneous infusion group were instructed in

self-management of the pump, as well as in needle insertion and care of the infusion site.

All subjects were monitored weekly with partial thromboplastin time determinations and complete blood counts. They were all counseled regarding signs and symptoms of complications. These included instructions to monitor urine color, to note any change in heart rate or feeling of vertigo, to report any signs or symptoms of infection at the injection site, and to report any unexplained bruising, multiple petechiae, or any bleeding that was difficult to stop.

Statistical analysis was performed with Barlett's test for homogenity of group variances performed for gestational age at onset and discontinuation of therapy, the amount of heparin infused per day, days of therapy, and measured partial thromboplastin times. Paired t tests were performed to evaluate the significance of differences, and Fisher's exact test was used to compare the complication rates in both groups. A p value of <0.05 was considered significant.

Results

As shown in Table I, comparison of the demographic characteristics such as age, race, parity, and diagnosis necessitating heparin therapy revealed no significant differences between the two groups. The gestational age at onset of therapy and total days of treatment also were not significantly different (Table II). These data reveal that the amount of heparin infused per day was significantly increased in the continuous infusion group (p < 0.05). The mean partial thromboplastin times noted in the continuous infusion group (20.6 ± 7.1) seconds) compared with the intermittent injection

group (10.4 ± 6.0 seconds) were significantly increased (p < .007).

The sites of administration in the continuous infusion group were rotated primarily between the abdomen and anterior thigh; 3 of the 8 patients used the deltoid region of the arm. No major complications were noted in the continuous infusion group; however, ecchymoses were reported in three patients, a small hematoma occurred in one woman, and oozing from the infusion site was seen in one subject. A nonpersistent epistaxis was noted in one patient who had noted ecchymosis. None of these complications was believed to be significant enough to cause discontinuation of therapy. The intermittent subcutaneous injection group was noted to have multiple episodes of minor side effects and two major complications—one injection site abscess and one large hematoma (Table II). The complication rates did not reach statistical significance, and no patient in either group experienced a recurrent embolic event.

Comment

Anticoagulation therapy by continuous subcutaneous infusion via external infusion pump appeared to be both safe and effective in this small group of patients. The successful use of this mode of administration is not consistent with the findings of the study performed in 1985 by Barss et al.11 In that study six patients received continuous subcutaneous infusion to reach a therapeutic partial thromboplastin time of 1.5 to 2.0 times control values. There were no recurrent thromboses, but five of the six patients experienced major or minor bleeding complications. This disparity may be secondary to a difference in infusion pumps used or type of infusion set. The infusion set used in this study did not involve an indwelling needle. Also, reliability, accuracy, and safety of the new infusion pump have been improved, compared with the unit used in the earlier study. Finally, the home nursing visits and intensive patient education may have played an important role in the different outcomes noted in the current study.

The significantly increased amount of heparin infused in patients by means of the infusion pump, noted in Table II, provided more adequate anticoagulation as measured by mean partial thromboplastin time. The improved anticoagulation profiles were obtained in these patients without an increase in serious morbidity. A central reason for these salutary results may be increased patient compliance. As previously noted, the

compliance rate of women using intermittent subcutaneous injection has been noted to be poor in previous studies.1, 2, 4 Patient acceptance of the soft catheter (vs the indwelling needle) and the reduced number of injections has been excellent in the patients who have used the continuous infusion pump. The increased cost of the subcutaneous infusion pump when compared with intermittent subcutaneous injection may be justified by these findings, which should lead to improved

Administration of heparin with the continuous subcutaneous infusion pump may be helpful not only in the context of heparin treatment for thromboembolism-related disorders but also for other complications necessitating anticoagulation during pregnancy. These indications include patients with atrial fibrillation, prosthetic heart valves, or valvular disease with a history of systemic emboli. 12, 15 The treatment regimen as outlined in this study appears to offer successful, safe, and efficacious anticoagulation and warrants further evaluation in pregnancy.

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Placental pathologic findings in preterm birth

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Microscopic features of placentas from 539 consecutive preterm deliveries and 214 term deliveries were compared. The presence of either umbilical or chorionic vasculitis was identified in 38% of the cases at 22 to 28 weeks' gestation, in 32% of the cases at 29 to 32 weeks' gestation, in 13% of the cases at 33 to 36 weeks' gestation, and in 10% of the cases at term (p < 0.0001). Decidual vascular abnormality was present in 70% of the cases at 22 to 28 weeks' gestation, in 35% of the cases at 29 to 32 weeks, in 29% of the cases at 33 to 36 weeks, and in 15% of the cases at term (p < 0.0001). Chronic villitis was significantly more frequent in preterm deliveries without umbilical vasculitis than in those cases with umbilical vasculitis (17% vs 8%, p < 0.05). Our data indicate that the placental lesions of umbilical-chorionic vasculitis, decidual vascular abnormality, and chronic villitis are related to preterm birth. Umbilical-chorionic vasculitis reflects acute ascending bacterial infection. Decidual vascular abnormality has been associated with maternal autoimmune or alloimmune disorders. Chronic villitis may indicate either congenital viral infection or maternal-fetal immunopathologic conditions. Both decidual vascular abnormality and chronic villitis may reflect the activation of inflammatory mechanisms capable of leading to preterm delivery. (Am J Obstet Gynecol 1991;165:934-8.)

Key words: Preterm delivery, vasculitis, villitis, placental pathologic findings

Preterm birth remains the primary cause of perinatal morbidity and mortality in this country.1 Certain maternal risk factors have been identified, such as multiple birth, incompetent cervix, lack of prenatal care, low socioeconomic status, and history of past preterm birth, but the mechanism by which they predispose to premature delivery is generally either unknown or related to an increased risk of ascending intrauterine infection.2 Our study was undertaken to search for placental pathologic processes that are significantly related to preterm birth and might provide clues to mechanisms for preterm birth. Acute ascending infection has been associated with increased incidence of prematurity, via mechanisms related to bacterial induction of cytokine and prostanoid production.2 Chronic immunologic or inflammatory conditions could elicit preterm labor via mechanisms similar to those proposed in cases with acute ascending infection. We hypothesized that placental lesions reflecting such chronic inflammatory processes are associated with prematurity. Chronic villitis, a chronic inflammation of the placental villi, indicates either congenital viral infection or maternal-fetal immunologic abnormality.³ Pathologic changes of the decidual vessels also have been suggested to have a basis in maternal autoimmune or alloimmune abnormality.⁴ Chronic intrauterine inflammation based at the level of either villus or decidua may alter collagen production by fibroblasts,⁵ leading to an increased likelihood of premature rupture of membranes. Finally, aberrant maternal-fetal immune interactions in the implantation site may lead to decidual vascular abnormality and an increased incidence of abruptio placentae.

To test this hypothesis, relationships between histologic evidence of acute ascending (bacterial) infection, chronic villitis, decidual vascular abnormality, and other disorders of the uteroplacental interface were examined in 539 cases of preterm birth and compared with findings in a control population of 214 spontaneous deliveries at term.

Material and methods

Between July 1983 and October 1990, all placentas from pregnancies delivered at <37 weeks' gestation at the Danbury Hospital were submitted for gross and microscopic examination by a single pathologist (C.M.S.). Cases of multiple gestation, stillbirth, congenital anomaly, maternal diabetes mellitus, preeclampsia, chronic hypertension, and elective delivery for indications such as ultrasonographically diagnosed intrauterine growth retardation were excluded from the consecutive series, yielding 539 cases of preterm birth. These included 241 cases of preterm labor, 94 cases of premature rupture of membranes, and 103 cases with

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history of both preterm labor and premature rupture of membranes. A clinical history of abruptio placentae was present in 65 cases, including 25 cases in which the patients presented in preterm labor. During the same period, 214 spontaneous vaginal deliveries at term in which placental abnormality also occurred were chosen at random and used as a control population. These included two cases with a history of preterm labor, 34 cases of rupture of membranes prior to the onset of labor, and two cases with a clinical history of abruptio placentae, for a total of 38 of 214 cases (18%).

Placental examination was performed according to a modification of Benirschke's method.6 Placentas were weighed after removal of adherent blood and umbilical cord. Presence, number, and estimated volumes of infarctions were recorded and gross diagnoses were confirmed microscopically. Placental infarction was defined microscopically as a lesion in which trophoblast, stroma, and vessels were uniformly necrotic. Placental infarct(s) were defined as gross lesions that were typically wedge shaped and based on the maternal surface. Four sections of grossly normal placenta were examined for the presence of chronic villitis.7,8 For the purpose of this study, the presence of chronic villitis was graded as absent or present. The presence of umbilical and chorionic vasculitis was determined by examining two sections of the umbilical cord and at least one section of chorionic plate including three chorionic vessels. The presence of umbilical-chorionic vasculitis was used as the discriminator between cases with and without a significant acute ascending infection. Decidual vascular abnormality included any of the following: decidual thrombosis, lack of physiologic change or fibrinoid necrosis of decidual vessels, and chronic decidual vasculitis involving vessels of the implantation site. This chronic inflammatory lesion is easily distinguished from the acute vasculitis associated with ascending bacterial infections by the nature of the inflammatory infiltrate (chronic mononuclear vs acute polymorphonuclear) and its location (basal plate vs parietal decidua). Decidual vessels were searched for in the four random sections of the placenta. If decidual vessels were not identified, these cases were excluded from pertinent analyses, and determinations of significance regarding the association of decidual vascular abnormality with preterm birth were made with the subgroups of those cases in which the decidual vessels were able to be assessed. Specifically, the number of cases in which decidual vessels were not able to be assessed was as follows: at the gestational age of 22-28 weeks, 3 of 26 cases (11.5%); at 29 to 32 weeks, 15 of 92 cases (16%); at 33 to 36 weeks, 49 of 421 cases (11.6%); and at 37 to 42 weeks, 0 of the 214 cases. The diagnosis of abruptio placentae was made by any or all of the following pathologic findings: adherent retroplacental clot or placental de-

pression and microscopic changes including disruption of basal plate with decidual necrosis and villous infarct, focal villous stromal hemorrhage, focal villous edema, and trophoblast basophilia. It is recognized that clinical abruptio placentae, histologically reflects a premature placental separation, and the pathologic diagnosis of such a process in the delivered (and completely separated) placenta is problematic. For the purposes of this study, the broadest set of pathologic criteria was used.

Gestational ages were calculated from the last menstrual period with modification by data provided by early ultrasonography or by ultrasonographic estimate alone when menstrual dates were uncertain. For the purpose of data analysis, gestational ages were grouped as 22 to 28 weeks, 29 to 32 weeks, 33 to 36 weeks, and term. Contingency tables examined the associations of placental pathologic condition and gestational age. The contributions of umbilical-chorionic vasculitis, chronic villitis, and decidual vascular abnormality to preterm birth were considered significant at p < 0.05.

Results

The incidences of the clinical presentations in the preterm and control population are presented in Table I. History of preterm labor, premature rupture of membranes, and/or abruptio placentae was identified in all of 26 cases (100%) at 22 to 28 weeks, 87 of 92 cases (95%) at 29 to 32 weeks, 367 of 421 (87%) cases at 33 to 36 weeks, and 38 of 214 (18%) at 37 to 42

The incidences of umbilical-chorionic vasculitis, chronic villitis, and decidual vascular abnormality throughout gestation are presented in Table II and Fig. 1. Umbilical-chorionic vasculitis was observed in 10 of 26 (38%) of deliveries between 22 and 28 weeks' gestation, 29 of 92 (32%) between 29 and 32 weeks' gestation, 54 of 420 (13%) between 33 and 36 weeks' gestation, and 22 of 214 (10%) between 37 and 42 weeks' gestation. The association of umbilical-chorionic vasculitis with lower (earlier) gestational age was highly significant (p < 0.0001).

Chronic villitis was observed in 1 of 26 (4%) deliveries between 22 and 28 weeks' gestation, 8 of 92 (9%) between 29 and 32 weeks' gestation, 67 of 421 (16%) between 33 and 36 weeks' gestation, and 51 of 214 (23%) between 37 and 42 weeks' gestation. Chronic villitis was significantly more frequent in preterm cases without umbilical-chorionic vasculitis than in those cases with umbilical vasculitis (71/445 [16%] vs 5/93 [5%], p < 0.01).

Decidual vascular abnormality was observed in 16 of 23 (70%) deliveries between 22 and 28 weeks' gestation, 27 of 77 (35%) between 29 and 32 weeks' gestation, 107 of 372 (29%) between 33 and 36 weeks' gestation, and 33 of 214 (15%) between 37 and 42 weeks' gestation

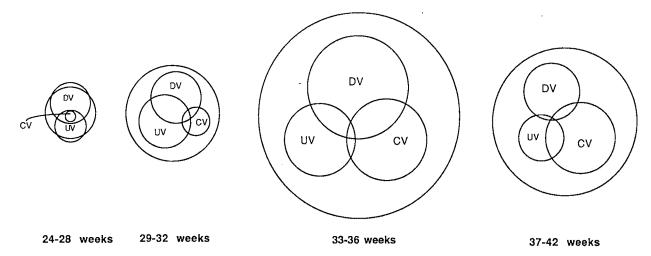


Fig. 1. Relative incidence of umbilical-chorionic vasculitis (UV), chronic villitis (CV), and decidual vascular abnormality (DV) as function of gestational age.

Table I. Incidence of documented clinical presentations* throughout four gestational age groups

	22-28 wk		29-32 wk		33-36 wk		37-42 wk	
	No.	%	No.	%	No.	%	No.	%
Preterm labor	20/26	77	57/92	62	267/421	63	2/214	0.09
Premature rupture of membranes	6/26	23	33/92	36	158/421	38	34/214	16
History of abrup- tio placentae	7/26	27	18/92	20	40/421	10	2/214	0.09
None of above	0/26	0	5/92	5	54/421	13	133/214	62

^{*}Cases may have had multiple clinical presentations (see text).

Table II. Incidence of umbilical-chorionic vasculitis, chronic villitis, and decidual vascular abnormality throughout four gestational age groups

	22-28 wk		29-32 wk		33-36 wk		37-42 wk	
	No.	%	No.	%	No.	%	No.	%
Umbilical-chorionic vas- culitis ($p = 0.0001$)	10/26	38 -	29/92	32	54/420	13	22/214	10
Chronic villitis $(p = 0.0001)$	1/26	4	8/92	9	67/421	16	51/214	23
Decidual vascular abnormality ($p = 0.0001$)	16/23	70	27/77	35	107/372	29	33/214	15

^{*}Decidual vessels could not be identified in all cases.

(p < 0.001). To test for independence of effects of umbilical-chorionic vasculitis and decidual vascular abnormality, contingency tables according to Fisher's exact test showed the effects of decidual vascular abnormality and acute inflammation on preterm birth to vary with gestational age. At 22 to 28 weeks decidual vascular abnormality and acute inflammation were strongly negatively correlated (p < 0.0001). Of this group there were 23 cases in which both umbilical-chorionic vasculitis and decidual vascular abnormality could be assessed. Three of 16 (19%) with decidual vascular ab-

normality also had umbilical-chorionic vasculitis, whereas 3 of 10 with umbilical-chorionic vasculitis had decidual vascular abnormality (p < 0.0005) and 7 of 23 had umbilical-chorionic vasculitis only (p < 0.0005). At 29 to 32 weeks a significant positive correlation was observed; only 11 of 27 cases with decidual vascular abnormality had umbilical-chorionic vasculitis and 11 of 29 cases with umbilical/chorionic vasculitis had decidual vascular abnormality (p < 0.01). No significant interactions were identified in the other gestational age groups.

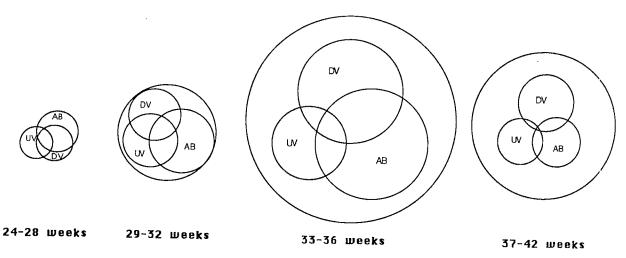


Fig. 2. Association of abruptio placentae (AB), umbilical-chorionic vasculitis (UV), and decidual vascular abnormality (DV).

Table III. Incidence of abruptio placentae and infarct throughout four gestational age groups

	22-28 wk		29-32 wk		33-36 wk		37-42 wk	
	No.	%	No.	%	No.	%	No.	%
Abruptio placentae $(p = 0.0001)$	13/26	50	41/92	45	119/421	28	25/214	12
Infarction $(p = 0.29)$	1/26	4	6/92	7	49/421	12	26/212	12

Given the hypothesis that chronic villositis and decidual vascular abnormality may have related immunopathologic origins, associations between these two processes were examined. At no gestational age was the presence of chronic villitis and decidual vascular abnormality significantly associated.

Abruptio placentae and placental infarcts may result from decidual vascular abnormality. In such instances the decidua may be so damaged as to preclude the actual assessment of the decidual vessels themselves. Of the 67 cases of preterm birth in which decidual vessels could not be assessed, 33 (49%) showed histologic evidence of abruptio placentae. Therefore the incidences of abruptio placentae and infarct were examined (Table III) as additional markers of decidual vascular pathology. Abruptio placentae, as defined in this study, was significantly more frequent in gestations of 22 to 28 weeks (13/26, 50%) as compared with term (25/214, 12%; p < 0.0001). The incidence of placental infarct did not vary significantly throughout gestation, ranging from 4% to 12%.

Clinical abruptio placentae is reported to occur in significantly increased frequency in association with acute inflammation,9 the associations of abruptio placentae as defined histologically, umbilical-chorionic vasculitis, and decidual vascular abnormality also were examined (Fig. 2). No significant interactions between umbilical-chorionic vasculitis, decidual vascular abnormality, and abruptio placentae were noted. Only decreasing gestational age was significantly related to increasing risk of histologic abruptio placentae (p < 0.001). Finally, the correlation between abruptio placentae diagnosed clinically and abruptio placentae defined histologically was examined. Overall, the clinical diagnosis of abruptio placentae was made in 65 to 539 (12%). Abruptio placentae was diagnosed in 7 of 26 (27%) deliveries at 22 to 28 weeks, 18 of 92 (20%) deliveries at 29 to 32 weeks, and 40 of 421 (10%) deliveries at 33 to 36 weeks. Thirty-three of the 65 cases (51%) of clinical abruptio placentae also had histologic evidence. Histologic changes considered to reflect abruptio placentae were diagnosed in 13 of 26 (50%) gestations at 22 to 28 weeks, 41 of 92 (45%) at 29 to 32 weeks, and 115 of 421 (28%) at 33 to 36 weeks. Of the 169 cases of histologic abruptio placentae, the clinical diagnosis of abruptio placentae was made in 33 (19%).

Comment

The data suggest that three separate placental lesions, umbilical-chorionic vasculitis, decidual vascular abnormality, and chronic villitis, are related to preterm birth. All three lesions may represent either acute or chronic inflammatory processes that could causally

be related to preterm birth via various mechanisms. In the case of acute intrauterine infection, generally caused by transcervical ascent of bacteria, the antigenic stimulus initiating the immune cascade of cytokines and prostanoids resulting in labor is exogenous (bacterial) in origin. In decidual vascular abnormality and chronic villitis, an antigenic stimulus may be viral or may arise from the maternal tissues (in autoimmune disorders) or from the fetoplacental unit itself (in alloimmune disorders), inciting chronic inflammation and leading to the respective lesions. The rarity of chronic villitis in early preterm pregnancies may be related to the immaturity of the placental macrophage.10 It is important to recognize that inflammation in the placenta may be represented by different lesions, ranging from simple edema to chronic villitis, depending on the gestational age and degree of functional maturity of the placental macrophage. Chronic maternal-fetal immunologic abnormality at early gestational ages may be reflected only in decidual (implantation site) abnormality. Precocious maturation of immature macrophages may be elicited by cytokines in vitro.11 The observation of chronic villitis at early gestational ages in association with cytomegalovirus infection may indicate a precocious maturation of the placental macrophage in association with maternally or fetally derived cytokines generated in response to the viral stimulus.

Further studies are required to clarify the mechanisms that lead to the placental lesions of decidual vascular abnormality and chronic villitis. Decidual vascular abnormality is recognized to accompany preeclampsia and maternal disease states such as diabetes mellitus, chronic hypertension, and autoimmune disorders, but our data demonstrated frequent decidual vascular abnormality in preterm births of women without evidence of any medical or hypertensive disease. Three placental lesions, umbilical-chorionic vasculitis, decidual vascular abnormality, and chronic villitis, could explain the cause of preterm birth in 96% of births between 22 and 28 weeks, 54% of cases between 29 and 32 weeks, and 46% of cases between 33 and 36 weeks. Current interventions designed to detect and eradicate vaginal pathogens will probably affect less than half of the pregnancies delivered between 24 and 28 weeks' gestation, an age at which risk for short- and long-term morbidity is great. Given the progressive shrinking of medical financial resources, placental examination would appear to be a key factor that may improve targeting of diagnostic and intervention modalities to those who would most benefit. In this study all cases of preterm birth were considered as a group. The further

description of the placental abnormality associated with specific clinical entities, such as preterm labor and premature membrane rupture, is the subject of a separate report.

Finally, in a low-risk community hospital population, abnormalities of the decidual vascular adaptation to pregnancy were identified in 183 of 539 cases (34%) of preterm delivery compared with 115 of 539 cases (21%) of umbilical-chorionic vasculitis. These data may underrepresent the contribution of decidual vascular abnormality to preterm birth, because 33 of the 67 cases (49%) in which decidual vessels could not be assessed had histologic evidence of abruptio placentae. These findings confirm the observations of Fox,12 who observed a 30% correlation of history of abruptio placentae with the presence of a retroplacental hematoma and a 35% correlation of the presence of a retroplacental hematoma with a clinical history of abruptio placentae. In such a low-risk population abnormalities of the placentation process, potentially of an immune etiology, are common correlates of premature birth.

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Transfer of aspirin across the perfused human placental cotyledon

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Pregnancy-induced hypertension is associated with a reduction in prostacyclin synthesis that is relative to normotensive pregnancy, whereas thromboxane A2 synthesis is unchanged or increased. The net effect is a decreased prostacyclin/thromboxane ratio that may result in the reduced fetal-placental blood flow seen in pregnancy-induced hypertension because thromboxane is known to constrict this circulation, Low-dose aspirin (acetylsalicylic acid), which is used to treat pregnancy-induced hypertension, selectively inhibits thromboxane synthesis and therefore may alter fetal-placental blood flow. We have investigated the transfer of acetylsalicylic acid in the perfused human placental cotyledon and its effects on fetal-placental perfusion pressure. Human placental cotyledons were perfused with tissue culture medium 199 plus 5% polyvinylpyrrolidone that was gassed with 95% oxygen/5% carbon dioxide at flow rates of 10 ml/min (maternal) and 4 ml/min (fetal). Acetylsalicylic acid (10⁻⁵ mol/L) was added to the maternal circuit, and cotyledons were perfused for 1 hour with aliquots taken from a closed fetal circuit every 5 minutes. Acetylsalicylic acid was assayed by spectrofluorometry at 306/412 nm. Our data indicate an initial rapid transfer of ascetylsalicylic acid during the first 5 minutes into the fetal-placental circulation, the concentration then decreased to a steady state at 0.4 × 10⁻⁵ mol/L. Resting perfusion pressure of both maternal and fetal circulation did not change after the addition of acetylsalicylic acid to maternal perfusate and transfer to the fetal circulation. (AM J OBSTET GYNECOL 1991;165:939-44.)

Key words: Low-dose aspirin, placenta, transfer, prostacyclin, preeclampsia

Pregnancy-induced hypertension and preeclampsia are associated with a reduction in uterine blood flow that sometimes leads to intrauterine growth retardation, as well as an increase both in platelet activation and the formation of placental and platelet thromboxane A2,1-5 a potent platelet aggregatory agent6.7 and vasoconstrictor. Although normotensive pregnancy is characterized by an increase in synthesis of vascular prostacyclin,8-10 which is a vasodilator and potent inhibitor of platelet aggregation,11 pregnancy-induced hypertension and preeclampsia are associated with a generalized reduction in prostacyclin synthesis. The reduced prostacyclin/thromboxane ratio of pregnancyinduced hypertension and preeclampsia may therefore result in the hypertension and reduced uterine and fetal-placental blood flow seen in these conditions. Lowdose aspirin (acetylsalicylic acid) may normalize the prostacyclin/thromboxane ratio in pregnancy-induced hypertension and preeclampsia by selectively inhibiting platelet and placental thromboxane and by leaving endothelial prostacyclin production unchanged. Selective inhibition of thromboxane in platelets may inhibit their aggregation in the uteroplacental circulation and may increase blood flow.

Pregnancies complicated by pregnancy-induced hypertension, preeclampsia, and intrauterine growth retardation have a high rate of abnormal systolic/diastolic ratios in the umbilical circulation, which indicates increased fetal-placental vascular resistance and reduced flow. Preliminary studies that used low-dose acetylsalicylic acid (1 to 2 mg/kg/day) suggest a reduction in both the severity and the incidence of pregnancy-induced hypertension^{12, 13} and intrauterine growth retardation, ^{14, 15} as well as an improvement in the systolic/diastolic ratio. In addition, low-dose acetylsalicylic acid has been shown to enhance the pregnancy-acquired refractoriness to angiotensin II in normotensive patients by altering vascular reactivity. ¹⁶

There is a lack of knowledge about the fetal concentration of acetylsalicylic acid that is reached with maternal ingestion of low-dose acetylsalicylic acid and the effects on the fetal-placental circulation. This is of particular interest, because fetal-placental vascular reactivity is, at least in part, mediated by prostaglandins.^{17, 18} The purpose of this in vitro study was

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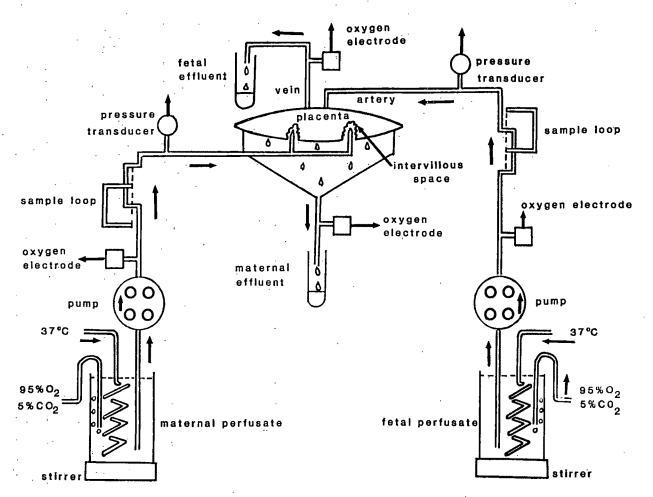


Fig. 1. Dually perfused human placental preparation.

twofold: to determine the transfer kinetics of low-dose acetylsalicylic acid in reaching the fetal circulation of the placenta when acetylsalicylic acid is infused into the intervillous circulation and to determine changes in perfusion pressure in the fetal circulation of the placenta under the influence of low-dose acetylsalicylic acid.

Material and methods

Eleven placentas were collected from normal term vaginal or cesarean section deliveries and were immediately transported to the perfusion laboratory.

The technique of perfusion of the isolated human placental cotyledon is essentially similar to that which we have previously described. Perfusion of the fetal placental circulation was begun at 4 ml/min with tissue culture medium 199 containing 5% polyvinylpyrrolidone 40, 0.1% bovine serum albumin, 48 µg/ml gentamicin, 20 IU/ml heparin; the medium was gassed with 95% oxygen and 5% carbon dioxide at 37° C. Perfusion on the maternal side was then begun at 10 ml/min with the perfusate described. The perfused

cotyledon was placed with the maternal surface downward over a plexiglas cone into which venous effluent from the intervillous space was allowed to drain. Excess nonperfused tissue was then trimmed from around the perfused cotyledon. Examination of the tissue after perfusion was begun revealed uniform blanching. Fetal and maternal effluents were measured to ensure that no perfusion mismatch occurred.

Lateral pressure was measured continuously with three pressure transducers; one was attached to the maternal line and two were attached to the fetal lines. Pressure was recorded on a computerized data acquisition system (IBM PS2, Armonk, N.Y.; Asystant Plus Software, Asyst Technologies, Rochester, N.Y.). Data were captured at a rate of 1 Hz. The entire apparatus including peristaltic pumps was housed in a cabinet maintained at 37° C by a thermostatically controlled electric fan heater (Fig. 1).

All perfusions were established within 20 minutes of placental delivery. Perfusion was continued for 20 minutes to allow the removal of debris, blood, and hypoxic metabolites before experimental manipulation. Oxy-



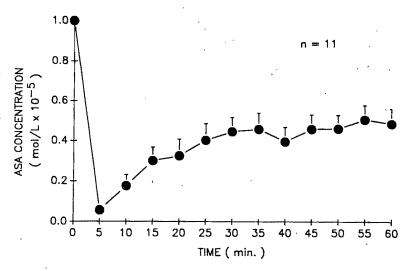


Fig. 2. Extraction of aspirin from maternal perfusate. Acetylsalicylic acid (ASA) (10⁻⁵ mol/L) was added to maternal perfusate and venous effluent was collected at 5-minute intervals and assayed for acetylsalicylic acid (mean \pm SEM; n = 11 experiments).

gen consumption was measured every 5 minutes to ensure placental viability. The pH of the perfusate was measured every 5 minutes and was maintained between 7.36 and 7.40.

After 20 minutes the maternal perfusate was changed to perfusion medium with the addition of acetylsalicylic acid to a final concentration of 10⁻⁵ mol/L, which corresponds to serum levels in women taking low-dose acetylsalicylic acid.20 At the same time the fetal perfusate was changed to a closed circuit containing 150 ml perfusion medium without acetylsalicylic acid. The venous effluent from the maternal side was collected every 5 minutes for 1 hour. Aliquots from the fetal circulation were similarly collected every 5 minutes for I hour. At the end of the experiment the perfused cotyledon was dissected out and weighed. All perfusate samples were centrifuged to remove cellular debris and were stored at -20° C. After all experiments were completed, the samples were thawed for analysis.

A standard curve of acetylsalicylic acid (10⁻⁹ to 10⁻⁵ mol/L) dissolved in perfusion medium that had previously been gassed with 95% oxygen and 5% carbon dioxide at 37° C was constructed (r = 0.964) by measuring the fluorescence on a luminescence spectrometer (LS-5B, Perkin-Elmer, Norwalk, Conn.) at 306/412 nm (excitation emission).21 All samples were then subsequently measured, and the acetylsalicylic acid concentration was computed.

Transplacental parameters were calculated as shown here.

Acetylsalicylic acid clearance (Clasa) was calculated on the basis of the formula:

$$Cl_{\text{ASA}} = \frac{C_{\text{F}} \cdot Q_{\text{F}}}{C_{\text{M}}}$$

where C_F is fetal venous concentration, Q_F is fetal flow

rate (4 ml/min), and C_M is maternal arterial concentration (10⁻⁵ mol/L).

Percent transfer was calculated on the basis of the formula:

Transfer (%) =
$$\frac{C_{\text{F}} \cdot Q_{\text{F}}}{C_{\text{M}} \cdot Q_{\text{M}}} \times 100$$

where Q_M is maternal flow rate (10 ml/min).

Permeability surface area product (P · S) was calculated by means of the formula:

$$P \cdot S = T/\Delta C$$

where T is net flux (moles per minute) and ΔC is maternal-fetal arterial difference.

Normalized permeability surface area product $(P \cdot S_N)$ was calculated according to the formula:

$$P \cdot S_{N} = \frac{P \cdot S}{\text{Wet weight of placenta (gm)}}$$

Multivariate analysis with Wilks' test was used to determine changes in perfusate acetylsalicylic acid concentrations over time.22

Results

There was a rapid extraction of acetylsalicylic acid from the maternal perfusate during the first 5 minutes of perfusion. The extraction rate then decreased and reached steady state by 25 minutes, when approximately 55% of the acetylsalicylic acid was extracted (Fig. 2).

There was a corresponding rapid rise of acetylsalicylic acid concentration in the fetal circulation during the first 5 minutes that reached 0.55×10^{-5} mol/L. The acetylsalicylic acid concentration then decreased slightly and reached steady state by 20 minutes at approximately 0.39×10^{-5} mol/L (Fig. 3). The accu-

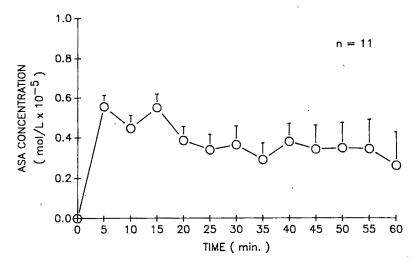


Fig. 3. Aspirin appearance in the tetal circulation. Acetylsalicylic acid (ASA) (10^{-5} mol/L) was added to maternal perfusate, and aliquots were collected every 5 minutes from a closed 150 ml fetal circuit and assayed for acetylsalicylic acid (mean \pm SEM; n=11 experiments).

Table I. Transplacental aspirin transfer parameters n = 11

Acetylsalicyclic acid parameters	$Mean \pm SE$		
Clearance, 5 min (ml/min)	2.22 ± 0.24		
Transfer 5 min (%)	22.19 ± 2.38		
Clearance, steady-state (ml/min)	1.54 ± 0.28		
Transfer, steady-state (%)	15.41 ± 2.75		
Permeability surface area produce (ml/min)	17.00 ± 1.78		
Normalized permeability surface area produce (ml/min/gm)	0.65 ± 1.31		

mulation of acetylsalicylic acid in the fetal circulation paralleled that of the apparent "maternal" extraction.

Acetylsalicylic acid clearance from maternal to fetal circulation was calculated to be 2.22 ml/min during the first 5 minutes; there was a 22% transfer of acetylsalicylic acid to the fetal circulation. Permeability surface area produce was 17 ml/min, and normalized permeability surface area produce was 0.647 ml/gm/min. At steady state acetylsalicylic acid clearance was 1.54 ml/min and the transfer from the maternal to fetal circulation was 15% (Table I).

There was obvious uptake of acetylsalicylic acid by the placental tissue. At steady state approximately 75% of the acetylsalicylic acid extracted from the maternal circulation (5.96 \times 10^{-8} mol/min) was taken up by placental tissue. When the placenta was perfused with 10^{-5} mol/L acetylsalicylic acid on the maternal side, concentrations in the fetal circulation did not exceed 0.55×10^{-5} mol/L.

There was no change in fetal perfusion pressure after the maternal circulation was perfused with 10^{-5} mol/L acetylsalicylic acid (Fig. 4).

Comment

Acetylsalicylic acid, as a result of its low molecular weight (180.2 gm), was able to cross the placenta rapidly; this is indicated by its rapid appearance in the fetal circulation during the first 5 minutes. The mean concentration of acetylsalicylic acid in the fetal circulation significantly increased over the first 5 minutes to reach a concentration of 0.55 \pm 0.06 \times 10⁻⁵ mol/L (mean \pm SEM), which was followed by a slight decrease with steady state reached by 20 minutes at a concentration of $0.39 \pm 0.07 \times 10^{-5}$ mol/L (mean \pm SEM). Multivariate analysis with Wilks' test showed significant overall change in acetylsalicylic acid concentration with time (p = 0.024). There was a significant increase in acetylsalicylic acid concentrations in the fetal circulation between 0 and 5 minutes. There was then a slight but significant reduction in acetylsalicylic acid concentration between 5 and 20 minutes (p < 0.0225) but there was no additional significant change after steady state

Multivariate analysis with Wilks' test again showed significant alterations in maternal acetylsalicylic acid concentrations with time (p = 0.047). There was a rapid significant extraction of acetylsalicylic acid from the maternal perfusate during the first 5 minutes of perfusion. The extraction rate then decreased and concentrations in the maternal venous effluent significantly increased by 25 minutes (p = 0.0012) with no additional significant change at steady state. At steady state approximately 60% of the acetylsalicylic acid was extracted, which left a concentration of $0.40 \pm 0.08 \times$ 10⁻⁵ mol/L in the maternal venous effluent. Rapid extraction of acetylsalicylic acid during the first 5 minutes was probably a result of the high concentration gradient between the two circulations; this resulted in the transfer of acetylsalicylic acid across the placenta. After 5

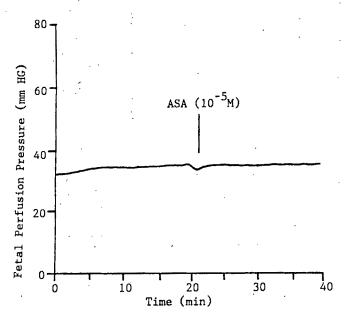


Fig. 4. Effect of addition of 10⁻⁵ mol/L acetylsalicylic acid (ASA) into maternal circulation of perfused placenta on fetal perfusion pressure. Data from typical experiment are shown.

minutes the concentration gradient was smaller and steady state was reached. The extraction of acetylsalicylic acid from the maternal perfusate parallels the appearance of acetylsalicylic acid in the fetal circulation.

At steady state the constant concentrations in both circulations suggest that the uptake of acetylsalicylic acid from the maternal circulation equals the metabolism of acetylsalicylic acid by the placenta. The loss of acetylsalicylic acid from the maternal circulation exceeded the accumulation of ascetylsalicylic acid in the fetal circulation by 75%, which presumably was a result of uptake and/or metabolism by the placenta. The results obtained for clearance, transfer, and permeability surface area product and its normalized derivative are consistent with other reports for substances with similar molecular weights and physical properties.23,24 Acetylsalicylic acid transfer (22% at 5 minutes and 15% at steady state) resulted in concentrations in the fetal circulation $(0.39 \times 10^{-5} \text{ mol/L})$ that were lower than those known to inhibit thromboxane and that have been shown not to affect prostacyclin synthesis in chorionic plate vessels in vitro.20 It is unlikely that low-dose acetylsalicylic acid given to pregnant women would alter fetal prostacyclin synthesis.25 In addition, the metabolism of acetylsalicylic acid in both fetal and maternal compartments is likely to lower the acetylsalicylic acid concentration further in the fetal circulation.

There was no change in fetal perfusion pressure after the maternal circulation was perfused with 10⁻⁵ mol/L acetylsalicylic acid. Although prostaglandins and thromboxane have been shown to mediate vascular reactivity in the perfused placenta, the effects are only manifest when tone'is induced in the system.18 If the fetal-placental circulation is maximally dilated at resting perfusion pressure, then alteration in prostaglandin synthesis might not produce an observable effect. The lack of change in fetal perfusion pressure during maternal infusion of acetylsalicylic acid supports this contention, which is in agreement with our previous observation¹⁷ that the cyclooxygenase inhibitor indomethacin did not affect resting perfusion pressure. However, if prostacyclin synthesis is important in regulating resting vascular tone, then we are observing a lack of inhibition of prostacyclin with this concentration of acetylsalicylic acid. Measurement of prostacyclin and thromboxane in the fetal-placental circulation during perfusion with acetylsalicylic acid will resolve this.

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Report of fourteen cases of nonimmune hydrops fetalis in association with hemorrhagic endovasculitis of the placenta

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Hemorrhagic endovasculitis of the placenta is a distinct vasodestructive process of unknown cause that has been associated with perinatal morbidity and mortality. A relationship between nonimmune hydrops fetalis and hemorrhagic endovasculitis has not been previously described. At a large teaching hospital, six cases of nonimmune hydrops fetalis were identified out of 72 cases of hemorrhagic endovasculitis over 6 years, for an incidence of 8%. Conversely, these same six cases represented 24% of the 25 cases of nonimmune hydrops fetalis from this time period. Eight additional cases of nonimmune hydrops fetalis were found among 2064 cases of hemorrhagic endovasculitis at the Michigan Placental Tissue Registry. In eight of the total 14 cases, after congenital malformations and cytomegalovirus infections were excluded, hemorrhagic endovasculitis was the only significant associated pathologic finding evident. The significance of the relationship between nonimmune hydrops fetalis and the vascular abnormalities of hemorrhagic endovasculitis remains to be determined. (AM J OBSTET GYNECOL 1991;165:945-50.)

Key words: Nonimmune hydrops fetalis, hemorrhagic endovasculitis, placental pathology, perinatal outcome

Hemorrhagic endovasculitis of the placenta is a distinct vasodestructive process of unknown cause first described in 1980.1 Its major histopathologic features include variable occlusion of chorionic vessels of all sizes by myointimal proliferation and mural and luminal thrombi in which fragmented erythrocytes and occasional nucleated red cells are entrapped. Mural nonexudative necrosis with hemorrhage into the surrounding villous stroma is also prominent, and a proliferative villous stromal response, referred to as hemorrhagic villitis, also may occur.2 A significant association between hemorrhagic endovasculitis and stillbirth, intrauterine growth retardation, and developmental abnormalities in surviving infants, including mental retardation, cerebral palsy, optic atrophy, hearing loss, and learning disabilities, has been reported.^{3,4} Nonimmune hydrops fetalis has been associated with various conditions; however, approximately 20% remain idiopathic.5,6 A relationship between the two disorders has not been previously described. A case of coexistence prompted our investigation into a possible association between these two entities.

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Material and methods

Medical records from January 1985 through December 1990 at William Beaumont Hospital, a large teaching hospital, were reviewed for both live births and stillbirths of infants with nonimmune hydrops fetalis in cases in which the placentas had been examined. All relevant placental histologic slides, routinely stained with hematoxylin and eosin, were then assessed for the presence of hemorrhagic endovasculitis by two independent examiners. The files of the anatomic pathology department were searched for all cases of hemorrhagic endovasculitis diagnosed among the total number of placentas examined during this same time period. These were subsequently reviewed for clinical evidence of nonimmune hydrops fetalis. The χ^2 test was used to test for an association between these two qualitative variables from the data acquired at this institution. Additionally, the files of the Michigan Placental Tissue Registry, a statewide referral center, were searched for cases of nonimmune hydrops fetalis among cases of hemorrhagic endovasculitis between 1982 and the first half of 1990.

Results

At William Beaumont Hospital, 3419 placentas from 33,838 deliveries were submitted for histologic examination during the 6 years spanning January 1985 through December 1990. Of these, 72 had microscopic features diagnostic of hemorrhagic endovasculitis. Six cases of nonimmune hydrops fetalis were identified out of these 72, for an incidence of 8%. Conversely, these same six cases of hemorrhagic endovasculitis representations.

Table I. Cases of nonimmune hydrops fetalis and hemorrhagic endovasculitis among placentas examined, 1985 to 1990

The state of the s	Hemorrhagic			
Nonimmune hydrops fetalis	Positive	Negative	Total	
Positive Negative TOTAL	6 66 72	19 3328 3347	25 3 394 3419	

Table II. Clinical and pathologic findings in nonimmune hydrops fetalis with hemorrhagic endovasculitis (14 cases)*

Case No.	Gestational age	Maternal age (yr)	Clinical information	Outcome	Hemorrhagic endovasculitis	Other
1	34 wk	33	Polyhydramnios, SGA, pulmonary hypoplasia, IRDS	Died at 20 hr	Moderate .	
2	34 wk	. 31	SGA, pulmonary hypo- plasia, Down syn- drome with ASD and VSD	Died at 11 days	Severe	,
3 .	27 wk	28	Pulmonary hypoplasia, IUGR, Turner pheno- type with cystic hy- groma	Stillborn	Focal	
4	36 wk	31	Polyhydramnios, pulmo- nary hypoplasia, IRDS	Died at 36 hr	Severe	
5	37 wk	32	Polyhydramnios	Survives at 1 yr	Focal	
6	28 wk	35	Polyhydrámnios	Stillborn	Moderate	Focal molar change
7	27 wk	40	Polyhydramnios	Stillborn	Severe	
8	"Near term"	.22	Focal hepatic vascular thrombosis	Stillborn	Severe	Mild acute placen- titis
9	"Near term"	30		Stillborn	Severe	
10	"Early 3rd tri- mester"	21	Polyhydramnios, IUGR	Stillborn	Moderate	
11	28 wk	27	Congenital cystic ade- nomatoid malforma- tion of lung	Neonatal death	Moderate	
12	36 wk	24	Polyhydramnios	Stillborn	Severe	CMV suggested, de- ciduitis and ab- scess
13	"1 mo premature"	Unknown	Polyhydramnios	Live-born, lost to follow-up	Focal	Active and chronic CMV infection
14	25 wk	. 27	IUGR, Turner syn- drome	Stillborn	Severe	Focal acute deciduo chorionitis, mar- ginal hematoma

SGA, Small for gestational age; IRDS, idiopathic respiratory distress syndrome; ASD and VSD, atrial septal defect and ventricular septal defect; IUGR, intrauterine growth retardation; CMV, cytomegalovirus.

sented 24% of the total 25 cases of nonimmune hydrops fetalis at this institution from this time period. With strict criteria, there was 100% interobserver agreement regarding the presence of hemorrhagic endovasculitis in the reviewed placental histologic slides. Additionally, with one exception, all were correctly diagnosed by the original reporting pathologists. The statistical processing of the results by χ^2 test shown in Table I provides support for a significant association between the finding of hemorrhagic endovasculitis with nonimmune hy-

drops fetalis (p < 0.001); however, this does not necessarily imply causality. The odds ratio, as an approximation of relative risk for nonimmune hydrops fetalis in the presence of hemorrhagic endovasculitis, is 15.9. An additional eight cases of nonimmune hydrops fetalis were found among the 2064 cases of hemorrhagic endovasculitis from a total of 12,916 placentas examined at the Michigan Placental Tissue Registry during the $8\frac{1}{2}$ years being considered. Clinical and pathologic findings in the 14 cases of nonimmune hydrops fetalis

^{*}Cases 1 to 6 from William Beaumont Hospital; cases 7 to 14 from Michigan Placental Tissue Registry.



Fig. 1. Obstetric ultrasonography of hydropic fetus from case 4. Cross section of thorax demonstrating marked pleural effusions (arrows) and thickened chest wall (arrowhead).

in association with hemorrhagic endovasculitis from both institutions are summarized in Table II. Of the total, there were eight stillbirths and four neonatal deaths. One infant survives, and one is lost to followup. The two examples which follow are representative of some of the clinical and pathologic features of these cases.

Case reports

Case 4. A female infant was delivered at 36 weeks' gestation by cesarean section of a 31-year-old mother with gestational diabetes mellitus. An ultrasonographic examination performed at 32 weeks had revealed marked polyhydramnios, severe fetal hydrops, large pleural effusions, ascites, and a questionable pericardial effusion (Fig. 1). Isoimmunization was ruled out, the Kleihauer-Betke test was negative, there was no hemoglobinopathy, serologic findings for relevant infectious agents were not significant, and chromosomal analysis showed a 46,XX karyotype. At birth, the generally edematous 2700 gm infant was limp, apneic, and bradycardiac. She was successfully ventilated after bilateral thoracentesis. In spite of aggressive mechanical ventilation, she died at 36 hours of age of apparent respiratory failure. Postmortem examination disclosed bilateral severe pulmonary hypoplasia, hyaline membrane disease, interstitial emphysema, and a right pneumothorax, in addition to pleural and peritoneal effusions. The placenta showed severe hemorrhagic endovasculitis exclusively (Figs. 2 and 3).

Case 5. A female infant was delivered at 37 weeks' gestation by cesarean section of a 32-year-old mother. After a report of decreased fetal activity, an ultrasonographic examination at 35 weeks had revealed polyhydramnios, massive pleural effusions, ascites, and skin edema. Isoimmunization was ruled out, the Kleihauer-

Betke test was negative, no hemoglobinopathy was demonstrated, serologic findings and microbiologic cultures for relevant infectious agents were negative, and chromosomal analysis was reported as 46,XX. At birth the 3799 gm edematous infant showed decreased tone and respiratory distress that required endotracheal intubation and mechanical ventilation. Worsening bilateral pleural effusions improved after thoracentesis on the seventh day after birth. Echocardiogram at 8 days showed decreased ventricular contractility with an ejection fraction of 62%, and she required medical therapy for hypertension. The hydrops gradually improved, and she was extubated and subsequently discharged at 1 month of age in stable condition on a regimen of digoxin. At 5 months, the infant was no longer taking cardiac medication, and a repeat echocardiogram was normal. Placental examination had disclosed focal hemorrhagic endovasculitis and villous edema (Figs. 4 and 5).

Comment

The development of nonimmune hydrops fetalis previously has been related to a number of fetal, placental, and maternal disorders. The more common causative factors cited include fetal heart disease, other intrathoracic abnormalities, nonimmune anemia, fetal infection, and fetal circulatory obstruction.5,6 In eight of the 14 cases presented here, after exclusion of congenital malformations (including trisomy 21, Turner syndrome, Turner phenotype with cystic hygroma, and cystic adenomatoid malformation of the lung) and cytomegalovirus infections, hemorrhagic endovasculitis was the only significant pathologic finding evident. As the characteristic alteration in hemorrhagic endovasculitis involves the destruction of fetal placental blood

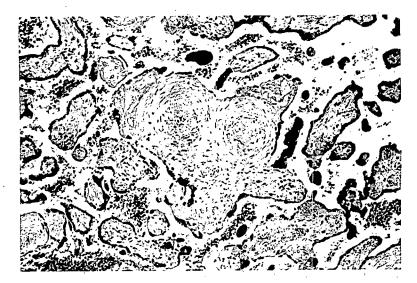


Fig. 2. Placenta with hemorrhagic endovasculitis from case 4. Near-total occlusion of three chorionic vessels of large central villus caused by proliferation of spindle cells. Note entrapped and fragmented red blood cells. Villus in lower right-hand corner exhibits features of hemorrhagic villitis. (Hematoxylin and eosin. Original magnification $\times 120$.)

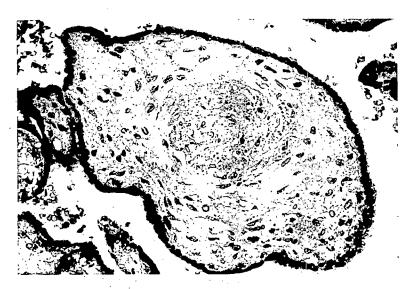


Fig. 3. Placental villus from case 4. Centrally located hemorrhagic endovasculitis—affected vessel with red blood cell fragments within its lumen and wall. (Hematoxylin and eosin. Original magnification ×300.)

vessels, red blood cell damage, enhanced coagulation, and intravillous hemorrhage, whether fetal blood loss by fetal-placental hemorrhage can be implicated in the development of hydrops fetalis in this setting is an interesting possibility that deserves further consideration. Alternatively, perhaps hypoxia may effect the fetal vascular damage that occurs in hemorrhagic endovasculitis, in which case placental ischemia as a possible etiologic factor in nonimmune hydrops fetalis deserves attention. Finally, there is always a possibility that both hemorrhagic endovasculitis and nonimmune hydrops

fetalis may be caused by a common, as yet undisclosed etiologic factor.

Statistical analysis was applied only to the results obtained from that part of the study conducted at William Beaumont Hospital and as such supports a significant relationship between hemorrhagic endovasculitis and nonimmune hydrops fetalis. Similar statistical testing was not possible with the data available from the Michigan Placental Tissue Registry; however, results from inquiry into its files were nonetheless enlightening. The disparate incidence of nonimmune hydrops fetalis in



Fig. 4. Placental villus with hemorrhagic endovasculitis from case 5. Chorionic vessels with narrowed lumina. Note diapedesis of red blood cells, some of which are fragmented, through wall of larger vessel. (Hematoxylin and eosin. Original magnification ×300.)

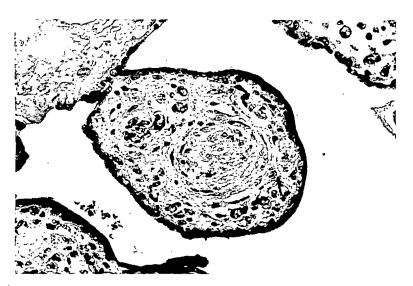


Fig. 5. Placental villus with hemorrhagic endovasculitis from case 5. Medium-sized vessel showing total occlusion by myointimal proliferation. Note entrapped red blood cells. (Hematoxylin and eosin. Original magnification ×300.)

association with hemorrhagic endovasculitis found at the two institutions involved may be explained by the highly selective nature of the cases submitted to the Michigan Placental Tissue Registry. Placentas are usually sent to this consultation center because of some characteristic of pregnancy or its outcome that is of special interest to the referring physician. Also, complete and relevant clinical information may not be as readily available in this situation.

This study supports the previously reported prognostic observation that there is an apparent doseresponse relationship between the severity of placental

involvement with hemorrhagic endovasculitis and pregnancy outcome.4 In the only two cases in which the infant survived beyond the neonatal period, focal, rather than diffuse, hemorrhagic endovasculitis was found. In the only other case where the placenta showed only focal involvement, the fetus was stillborn but also had multiple serious anomalies. The incidence of hemorrhagic endovasculitis varies from 0.67% of unselected pregnancies,8 to 2.1% among placentas submitted for examination at William Beaumont Hospital, to 19.4% at the Michigan Placental Tissue Registry. The incidence of hemorrhagic endovasculitis in unselected pregnancies might be higher than the reported 0.67%, for in the cited prospective study only two microscopic sections from each placenta were examined rather than more extensive sampling being done.

Finally, an appropriate investigation into the possible cause of nonimmune hydrops fetalis in a specific case is important not only for the immediate treatment of the neonate but also for the management of subsequent pregnancies. It has been reported that hemorrhagic endovasculitis, like nonimmune hydrops fetalis, carries a risk for recurrence.³ Future attention to the association of nonimmune hydrops fetalis and hemorrhagic endovasculitis hopefully will contribute to better understanding of the interrelationship between these entities

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Nifedipine pharmacokinetics and pharmacodynamics during the immediate postpartum period in patients with preeclampsia

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Pharmacokinetic and pharmacodynamic parameters of oral nifedipine were studied in the immediate postpartum period in eight women with preeclampsia. Peak serum concentrations of $18 \pm 2.1~\mu g/L$ occurred 40 minutes after ingestion of nifedipine (10 mg). The terminal elimination half-life (mean = 1.35 ± 0.3 hours) was found to be shorter than that reported for normotensive volunteers or nonpregnant hypertensive women (mean, 3.4 ± 0.4 hours). A mean apparent oral elimination clearance of $3.3 \pm 1.3~L/hr/kg$ was more rapid than that found in normal volunteers (mean, $0.49 \pm 0.09~L/hr/kg$) or in women with pregnancy-induced hypertension in the third trimester (mean, $2.0 \pm 0.8~L/hr/kg$). Initial nadirs in mean arterial pressure were noted at 50 minutes after ingestion of nifedipine, with an average reduction in mean arterial pressure of 13.8~mm Hg. A dosing interval of every 3 to 4 hours is suggested when rapid-release nifedipine is used in the postpartum patient with preeclampsia. (AM J OBSTET GYNECOL 1991;165:951-4.)

Key words: Nifedipine, pharmacokinetics, preeclampsia

Approximately 7% of all pregnancies are complicated by hypertension with preeclampsia; this is responsible for 70% of all hypertension occurring in pregnancy. The diagnosis of preeclampsia is based on the presence of hypertension and proteinuria or edema or both. In most patients the hypertension associated with preeclampsia appears to be a secondary effect of generalized vasospasm. Because of this associated vasospasm, antihypertensive agents with vasodilatory properties are very desirable. The current standard treatment for severe hypertension associated with preeclampsia is the intravenous administration of hydralazine.⁵

During the past several years many new antihypertensive agents have become available. One such agent, the calcium channel antagonist nifedipine, has potent activity against vascular and extravascular smooth muscle contractions. Dosage is usually initiated at 10 mg given orally three times a day. It has also been shown to promptly and effectively resolve elevated blood pressure in antepartum patients with pregnancy-induced hypertension, as well as severe preeclampsia in the postpartum period, but data are limited. The pharmacokinetics of nifedipine have been evaluated in non-

pregnant subjects, ¹⁰ in women during preterm labor tocolysis, ¹¹ and in women in the third trimester with pregnancy-induced hypertension. ¹² This investigation was designed to determine the pharmacokinetic disposition and pharmacodynamics of oral nifedipine during the immediate postpartum period in patients with preeclampsia.

Material and methods

Eight women with an intrapartum diagnosis of preeclampsia were invited to participate in the study, following guidelines established by the institutional review board at the University of Tennessee, Memphis. Preeclampsia was diagnosed on the basis of the criteria established by the Committee on Terminology of the American College of Obstetricians and Gynecologists.13 Patients were excluded from participation if their medical history revealed an adverse reaction to calcium channel blockers, if they had used a calcium channelblocking agent within 8 hours before the investigation, or if they required supplemental therapy with other antihypertensive agents during the course of the postpartum pharmacokinetic evaluation. All patients received standard therapy for preeclampsia in the postpartum state, including bed rest and a continuous infusion of magnesium sulfate at a dose required to maintain a serum concentration between 6 and 8 mg/dl. The primary intravenous fluid was 5% dextrose in lactated Ringer's solution, and total intravenous fluid intake was maintained at 100 ml/hr. Oral intake was not permitted during the study. After patients gave antepartum informed consent to participate in the pharmacokinetic evaluation, arterial catheters were

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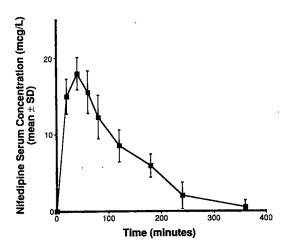


Fig. 1. Mean \pm SD serum drug concentration versus time after oral administration of 10 mg nifedipine.

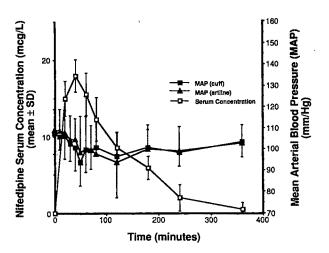


Fig. 2. Mean ± SD serum drug concentration and mean arterial blood pressure (MAP) versus time after oral administration of 10 mg nifedipine. artline, Intraarterial pressure measurement. cuff, Automated blood pressure cuff measurement.

placed in either the right or left radial artery for blood pressure monitoring and phlebotomy access. Once delivered and in the recovery room, the patients were placed in a supine position with the heads of the beds elevated to approximately 30 degrees. The arterial catheter was positioned and flushed, and an automated blood pressure monitor was placed on the patient's opposite arm. Blood pressure was measured at baseline and every 10 minutes for the first hour, then at 70, 80, 120, 180, 240, and 360 minutes after the study dose of nifedipine. A 5 ml blood sample was obtained immediately before oral intake of the study dose of 10 mg nifedipine. Postdose blood samples were obtained at 20, 40, 60, 80, 120, 180, 240, and 360 minutes. The samples were collected in subdued lighting, protected from light during transport, and centrifuged immediately after collection. The serum was stored at -70° C until analysis. Samples were analyzed by highperformance liquid chromatography. A Hewlett Packard 1084 chromatograph (Hewlett Packard, Avondale, Pa.) equipped with a variable wavelength detector was used for analysis. Separation was achieved with an LC-8DB, 15 cm \times 4.6 mm, 5 μ m column (Supelco, Inc., Bellefonte, Pa.). Acetonitrile and potassium phosphate buffer, 50 mm (55:45), was the mobile phase of choice. Peak detection was achieved at 236 nm, 0.008 AUFS (absorbance units full scale). Flow rate was 1 ml/min. Serum nifedipine concentrations were measured after extraction of nifedipine and chlorpropamide (internal standard) on Supelclean LC-8 SPE tubes (Supelco). One milliliter of serum was diluted to 5 ml with milli Q water (Millipore Inc., Bedford, Mass.), applied to preactivated cartridges, and eluted with 500 µl of tetrahydrofuran. The resulting eluant was dried in the dark under a stream of nitrogen gas. The residue was resuspended in 200 µl of mobile phase, and 20 µl was assayed for nifedipine concentration. The assay's lower limit of sensitivity was <5 ng/ml. Samples were run in duplicate.

The serum concentration versus time data from the β (terminal) elimination phase was fitted to a single exponential term (ke, the elimination rate constant) by use of a multilinear least-squares regression program. The terminal elimination half-life (t 1/2) was calculated from the equation:

$$t 1/2 = 0.693/ke$$

Noncompartmental analysis was used to estimate clearance and area under the serum concentration versus time curve, because this method does not assume the elimination data fit a chosen model. ¹⁴ The area under the serum concentration versus time curve was calculated by the trapezoidal rule. Apparent oral elimination clearance was expressed in relation to actual body weight and was calculated from the equation:

$$\frac{\text{Clo} = \text{dose per kg body weight}}{\text{AUC}_{0.6}}$$

where Clo is apparent oral clearance and $AUC_{0.6}$ is area under the concentration versus time curve from 0 to 6 hours.

Results

The eight patients all had preeclampsia and were in the immediate postpartum period. All patients had proteinuria, but none had evidence of chronic renal disease. In one patient the syndrome of hemolysis, elevated liver functions, and low platelets (HELLP syndrome) developed. Five of the eight patients received intravenous hydralazine in the antepartum period for systolic blood pressure >160 mm Hg or diastolic blood pressure >110 mm Hg. Nifedipine was well tolerated

Table I. Pharmacokinetic data

Patient No.	Weight (kg)	Dose (μg/kg)	Ke (L hr)	t½ (hr)	Clo (L/hr/kg)	Auc (μg·hr/L)	Cmax (µg/L)
1	46.0	217.4	0.441	1.57	5.8	37.2	17
2	85.5	116.9	0.432	1.60	2.5	46.4	17
3	95.5	104.7	0.573	1.20	2.2	46.6	21
4	108.2	92.4	0.396	1.74	2.1	43.5	17
5	76.8	130.2	0.720	0.96	4.4	29.4	19
6	85.9	116.4	0.659	1.05	2.6	44.7	21
7	89.5	111.7	0.605	1.14	3.2	35.1	16
8	58.6	170.6	0.441	1.56	3.9	43.8	18
Mean ± SD	80.8 ± 20.4	132.5 ± 41.4		1.35 ± 0.3	3.3 ± 1.3	40.8 ± 6.2	18.3 ± 1.9

Pharmacokinetic data, group means, and standard deviations after oral administration of 10 mg nifedipine. Ke, Elimination rate constant; t½, elimination half-life; Clo, apparent oral elimination clearance; Auc, area under the concentration versus time curve; Cmax, maximum serum concentration.

by all patients, with headache being the only adverse effect reported. Nifedipine was undetectable in eight of eight patients at time 0 and in six of eight patients at 360 minutes. Peak serum concentration occurred at 40 minutes, which was the timing of the second sample collection.

The mean serum concentration versus time graph is depicted in Fig. 1. All patients entered the terminal elimination phase at approximately 40 minutes after drug ingestion. Whereas patient concentrations were variable, the declining concentrations were fitted to a one-compartment model well, with a correlation coefficient (mean \pm SD) of 0.984 \pm 0.01. The pharmacokinetic parameters for each patient are described in Table I. The serum half-life (mean \pm SD) was 1.35 \pm 0.3 hours, with a range of 0.96 to 1.74 hours. Oral clearance (mean \pm SD) was 3.3 \pm 1.3 L/kg/hr, with a range of 2.1 to 5.8 L/kg/hr.

The initial mean arterial blood pressure averaged 107.5 mm Hg when taken by the automatic blood pressure cuff and 109.0 mm Hg when recorded from the arterial line, with an average during therapy of 100.7 and 101.1 mm Hg, respectively. Initial nadirs were noted at 50 minutes with an average of 93.7 and 98.3 mm Hg, respectively, correlating well with the timing of the peak serum concentration at 40 minutes (Fig. 2).

Comment

Nifedipine, a dihydropyridine derivative, is a calcium channel blocker with potent peripheral arterial vasodilating properties. The drug has been used in the successful treatment of hypertension since 1976. Extensive reviews leave indicate that single-dose nifedipine is associated with a 25% reduction in systolic blood pressure, diastolic blood pressure, and mean arterial pressure in 98% of cases. Walters and Redman⁶ reported excellent resolution of hypertension, without any adverse maternal or fetal effects, when treating pregnant women with severe preeclampsia. Barton et al. re-

ported a reduction in mean arterial blood pressure when treating hypertension associated with severe preeclampsia in the postpartum period. Furthermore, nifedipine has also been reported to improve renal function with a beneficial effect on urine output when treating preeclampsia in the postpartum period.⁹

Many pathophysiologic changes occur during pregnancy, preeclampsia, and in the postpartum period, which may affect drug disposition and ultimately be responsible for altered pharmacologic activity. In our patients a mean peak nifedipine serum concentration of $18 \pm 2.1 \,\mu g/L$ was achieved 40 minutes after a 10 mg oral dose. Previous studies of nifedipine after a 10 mg oral dose found peak serum concentration to be $38.6 \pm 1.8 \,\mu g/L$ at 40 minutes in women with pregnancy-induced hypertension in the third trimester¹² and $73.48 \pm 17.48 \,\mu g/L$ in nonpregnant normal subjects. 10 The difference may be due to enhanced firstpass elimination in our pregnant and postpartum population. Nifedipine is eliminated metabolically during the first pass through the liver and therefore only an estimated 60% to 70% of the dose enters the systemic circulation in nonpregnant patients.21 Furthermore, nifedipine has been shown to increase hepatic blood flow as a result of arterial vasodilatation.22 Another factor most likely to contribute to our patients' lower peak serum concentrations is the increased intravascular volume that results from fluid shifts during the postpartum period in patients with preeclampsia. The mobilization of fluid from the extravascular to the intravascular space increases the serum volume of distribution, therefore decreasing the highest serum concentration achieved.

Serum concentrations were undetectable in most patients at 360 minutes. This once again confirms lack of drug accumulation previously reported in pregnant patients with dosing every 6 hours^{11, 12} and raises concern over the adequacy of a 6- to 8-hour dosing interval.

The terminal elimination half-life was found to be 1.35 ± 0.3 hours, which confirms the findings of Fer-

guson et al., ¹¹ who found a half-life of 1.3 ± 0.43 hours in third-trimester women receiving nifedipine for to-colysis and Rogers et al., ¹² who found a half-life of 1.3 ± 0.5 hours in third-trimester patients with pregnancy-induced hypertension. This half-life is shorter than that reported in nonpregnant controls. ¹⁰ The shortened half-life appears to be due to the physiologic changes of pregnancy and not to factors exclusively associated with preeclampsia, the postpartum period, pregnancy-induced hypertension, or preterm labor.

The clearance of nifedipine was greater in our population (3.3 \pm 1.3 L/hr/kg) than in third-trimester women receiving nifedipine for pregnancy-induced hypertension (2.0 \pm 0.8 L/hr/kg). and in nonpregnant controls (0.49 \pm 0.09 L/hr/kg). The increased clearance of nifedipine in our patients appears to be due to the increased intravascular volume resulting from fluid shifts occurring during the postpartum period in patients with preeclampsia.

In summary, this study confirmed a shorter half-life and found a more rapid clearance and lower peak serum concentration for nifedipine in patients during the postpartum period with preeclampsia than in nonpregnant controls. Moreover, our results found a more rapid clearance and lower peak serum concentration for nifedipine in postpartum patients with preeclampsia when compared with third trimester women receiving nifedipine for pregnancy-induced hypertension. Finally, our results suggest that nifedipine may be efficacious in treating hypertension associated with preeclampsia in the postpartum period; however, because of the pharmacokinetic changes described, dosage, and dosing intervals (i.e., every 3 to 4 hours) may need to be altered. The rapid-release capsule is of vital importance when titrating the dose to the patient's individual response. Thereafter, the sustained release form of nifedipine may be more practical and may improve compliance.

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Microbiologic causes and neonatal outcomes associated with chorioamnion infection

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Chorioamnion infection is associated with histologic chorioamnionitis and prematurity, but the specific chorioamnion microorganisms associated with histologic chorioamnionitis, prematurity, and poor neonatal outcome have not been identified. Bacteria were recovered from the chorioamnion cultures of 32% of 112 placentas delivered at ≤34 weeks' gestation and from 19% of 156 placentas delivered at >34 weeks' gestation (odds ratio 2.1; 95% confidence interval 1.1 to 3.8). Chorioamnion bacteria most highly related to both prematurity and histologic chorioamnionitis were group B Streptococcus and Fusobacterium species. Chorioamnion infection with Peptostreptococcus was significantly related only to preterm delivery, and infection with Escherichia coli, Bacteroides, and Ureaplasma were significantly related to histologic chorioamnionitis. Among preterm infants, isolation of bacteria from the chorioamnion was related to an increased risk of neonatal death (rate ratio 3.8; 95% confidence interval 1.4 to 11.6). Bacterial infection of the chorioamnion is related to preterm birth, histologic chorioamnionitis, and neonatal death. (AM J OBSTET GYNECOL 1991;165:955-61.)

Key words: Chorioamnion infection, chorioamnionitis, neonatal mortality

Recent studies have shown a positive relationship between preterm delivery, chorioamnion infection, and histologic chorioamnionitis. Microorganisms are recovered 2 to 4 times more frequently from the chorioamnion of placentas delivered before term than those delivered at term.1-3 Histologic chorioamnionitis is detected in 19% to 74% of placentas delivered preterm compared with 4% to 16% of placentas delivered at term. 1, 4-6 Chorioamnion infection and histologic chorioamnionitis are closely related but not synchronous. Microorganisms have been recovered from 51% to 71% of placentas with histologic chorioamnionitis, compared with 23% to 45% of those without histologic chorioamnionitis.1,2,7,8 Prolonged labor or rupture of membranes may result in colonization of the upper genital tract by vaginal bacteria. However, chorioamnion infection was associated with preterm delivery in one study after these potential confounding factors were controlled by logistic regression.1

The microorganisms most commonly recovered from the chorioamnion include *Ureaplasma urealyticum*,

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facultative and anaerobic gram-positive cocci, Gardnerella vaginalis, and Bacteroides species. 1. 2. 6-8 Haemophilus influenzae, Neisseria gonorrhoeae, and Chlamydia trachomatis are rarely recovered from the placenta. 1. 8-10 The recovery of microorganisms from term placentas and from placentas without inflammation raises the possibility that only certain microorganisms are associated with preterm delivery and histologic chorioamnionitis. Too few subjects have been included in any one study to identify the particular microorganisms associated with preterm birth. Except for *U. urealyticum*, no specific microorganisms have been associated with histologic chorioamnionitis. 8

Amniotic fluid infection has been associated with neonatal respiratory distress syndrome and neonatal infectious morbidity.¹¹ Histologic chorioamnionitis has been associated with perinatal death, elevated antibody levels to *U. urealyticum*,⁸ and sepsis within 48 hours of birth.⁵ Neonatal outcome in pregnancies with chorioamnion infection has not been reported.

One purpose of this study was to confirm the relationship between chorioamnion infection and preterm delivery and histologic chorioamnionitis after accounting for potentially confounding labor variables. The second purpose of this study was to determine whether individual microorganisms infecting the chorioamnion were associated with preterm delivery and histologic chorioamnionitis. The third purpose was to determine whether chorioamnion infection was associated with neonatal death or respiratory distress syndrome.

Material and methods

Women were enrolled between October 1987 and March 1990 from the labor and delivery unit of University of Washington Medical Center. Women who agreed to participate in the study signed consent forms approved by the University of Washington Human Subjects Review Committee. All women admitted to the labor and delivery unit during weekdays were screened for eligibility. Women were excluded from enrollment if they were <16 years or >40 years of age, had documented uterine or fetal anomalies, hypertension, diabetes, placenta previa, abruptio placentae, cervical cerclage, or fetal distress, or had received antibiotics in the previous week. The number of women in preterm labor who were screened and excluded or approached for the study has been previously published.12 After a subject was enrolled, the next eligible control woman who was delivered within daytime working hours was enrolled.

The case group consisted of 146 women admitted to the hospital in preterm labor at a gestational age of 22 to 34 weeks and who were delivered at ≤34 weeks' gestation. We defined prematurity as a gestational age of ≤34 completed weeks because the prevalence of chorioamnion infection and histologic chorioamnionitis is stable at >35 weeks' gestation.1.5 Preterm labor was defined as regular painful contractions occurring at least every 10 minutes accompanied by progessive cervical dilatation or by a Bishop score >4 that included ≥50% cervical effacement and ≥1 cm dilatation. 18 Preterm premature rupture of membranes was defined as spontaneous rupture of membranes before the onset of contractions at a gestational age of 22 to 34 weeks. The control group consisted of 184 women without preterm labor who were delivered at >34 weeks' gestation and 74 women who were enrolled in preterm labor but were delivered at >34 weeks' gestation. Chorioamnion cultures and histologic samples were obtained from 112 (77%) of the women in the group of 146 cases, 35 (47%) of the 74 women enrolled in labor at ≤34 weeks' gestation who were delivered at >34 weeks, and 121 (66%) of the 184 controls. Only patients with both chorioamnion culture and placental histologic results were included in this report. The data for the two groups of women delivering at ≥34 weeks' gestation were similar with respect to chorioamnion infection and histologic chorioamnionitis, so the data were combined for a total of 156 control women. The median gestational age was 31 weeks for the case group and 39 weeks for the control group. Gestational age was estimated by the date of the mother's last menstrual period, fundal height, ultrasonography, and an evaluation of the newborn with a standardized Ballard examination.14

Social history and demographic data were collected

during a structured interview. Labor and delivery information was recorded from medical records with a standardized data coding sheet.

Delivered placentas were placed on a clean surface; the chorion and amnion were separated manually by gloved personnel. Care was taken to prevent contamination of the exposed surfaces. Sterile Dacron swabs were rolled across the internal surface of the separated membranes and used to inoculate culture media as previously described.¹ Placentas that could not be sampled immediately were stored at room temperature to prevent killing of anaerobic microorganisms at low temperatures. All placentas were cultured ≤24 hours of delivery.

The fetal membranes were grasped with forceps, and a 2 to 3 cm strip was cut from the point of rupture to the discoid placental margin to prepare a "membrane roll" for histologic examination. A diagnosis of histologic chorioamnionitis was made if ≥ 10 polymorphonuclear leukocytes were present per field in 10 nonadjacent $400 \times$ fields as previously described.

The association of chorioamnion infection or inflammation with neonatal outcome was assessed only in the case group because the preponderance of neonatal morbidity and mortality occurs among infants born at ≤34 weeks' gestation. These data were collected from the medical records of the mother and neonate. Follow-up information was obtained by telephone interview 6 weeks after delivery. Infant death included stillbirths and neonatal deaths occurring ≤28 days after birth. Respiratory distress syndrome was defined as the requirement of supplemental oxygen to prevent cyanosis, the clinical observation of dyspnea, and the presence of air bronchograms and a reticulogranular pattern shown by a chest radiograph.

To examine the statistical significance of the membrane isolates with delivery at ≤34 weeks' gestation or with histologic chorioamnionitis, we used the p value associated with a Yates' corrected χ^2 statistic or a twosided Fisher exact test.15 A p value of 0.05 was considered to be statistically significant. To describe the relationship of chorioamnion bacteria and histologic chorioamnionitis with delivery at ≤34 weeks' gestation, we used an odds ratio as a point estimate of the excess risk. The adjusted odds ratios were determined and evaluated for statistical significance by logistic regression analysis.16 To determine the statistical significance of the odds ratio, we used 95% confidence intervals verified by likelihood ratio tests. Among the neonates delivered at ≤34 weeks' gestation, proportional hazards models developed by Cox17 were used to estimate the rate ratio of neonatal death associated with chorioamnion inflammation or infection. The outcome was neonatal death and the time to event was the number of days of life between birth and death. The relationship

Table I. Association of chorioamnion bacteria and chorioamnionitis with preterm delivery at ≤	34
weeks' gestation	

, Chor	ioamnion	· Weeks' gestation at delivery			٧.
Bacteria*	Inflammation	≤ 34 $(n = 112)$	> 34 $ (n = 156)$	Odds ratio	95% CI
Present	Present	22	10	7.0	3.0-16.4
Absent	Present	44	25	5.6	3.0-10.6
Present	Absent '	14	19	2.3	1.1-5.2
Absent	Absent	32 (29%)	102 (65%)	Ref	ference

The following characteristics were evaluated for their importance as confounding variables: race, marital status, education, gravidity, prior preterm birth, prior spontaneous abortion, referral source, smoking, alcohol use, delivery method, bacterial vaginosis, premature rupture of membranes, hours of membrane rupture, and hours of labor. CI, Confidence in-

of chorioamnion inflammation or infection with respiratory distress syndrome was evaluated among the surviving neonates by using Mantel-Haenszel stratified analyses with a risk ratio to provide the point estimate of excess risks.18

Results

Bacteria were recovered from 36 (32%) of 112 placentas delivered at ≤34 weeks' gestation and from 29 (19%) of 156 delivered at >34 weeks' gestation (unadjusted odds ratio 2.1; 95% confidence interval 1.1 to 3.8). Two or more bacteria were recovered from 17 (15%) of 112 placentas delivered at ≤34 weeks' gestation and 12 (8%) of 156 placentas delivered at >34 weeks' gestation (p = 0.05). Genital mycoplasmas were not related to delivery at ≤34 weeks' gestation. The recovery of bacteria other than U. urealyticum was related to preterm delivery at ≤34 weeks' gestation with and without placental membrane inflammation (Table I). Chorioamnion inflammation was highly related to delivery at ≤34 weeks, with and without chorioamnion bacteria. Inflammation and bacterial infection of the chorioamnion were independently related to delivery at ≤34 weeks, but each was most strongly related to preterm delivery in the presence of the other. None of the characteristics listed in Table I evaluated by logistic regression models as potential confounding variables altered the magnitude or significance of the relationship of inflammation and bacterial infections of the chorioamnion with delivery at ≤34 weeks' gestation.

Group B Streptococcus, Peptostreptococcus, and Fusobacterium recovered from the chorioamnion were associated with a preterm delivery at ≤34 weeks' gestation. U. urealyticum was recovered from similar proportions of preterm and term placentas (Table II). Anaerobic bacteria were recovered more often from placentas delivered at ≤34 weeks' gestation (18%) than from those delivered at >34 weeks' gestation (5%) (odds ratio 5.8; 95% confidence interval 2.0 to 20.5). Peptostreptococcus and Fusobacterium species were each significantly related to preterm birth, and Bacteroides species were weakly related to delivery at ≤34 weeks' gestation. Because of the infrequency of their isolation, it was not possible to assess whether placental Mycoplasma hominis, Actinomyces, Mobiluncus, or Candida species were associated with preterm birth. A lack of vaginal contamination was suggested by the low frequency of Lactobacillus isolated from the chorioamnion cultures.

Histologic chorioamnionitis was detected in 66 (59%) of 112 placentas delivered at ≤ 34 weeks' gestation and 35 (22%) of 156 placentas delivered at >34 weeks' gestation (unadjusted odds ratio 5.0; 95% confidence interval 2.8 to 8.8). Because the relationship between chorioamnion infection and inflammation was similar among both groups (data not shown), data were combined (Table III). U. urealyticum (which was not related to preterm birth) was strongly related to histologic chorioamnionitis. Group B streptococci and Fusobacterium were related to both preterm delivery and inflammation of the chorioamnion. Bacteroides and Escherichia coli were related to histologic chorioamnionitis. However, Peptostreptococcus (which was related to preterm birth) was not related to histologic chorioamnionitis.

To assess the neonatal risks associated with chorioamnion infection, we determined the frequency of death and respiratory distress syndrome among the 112 infants delivered at ≤34 weeks' gestation. Sixteen (14%) of the 112 infants died (Table IV). Microorganisms other than U. urealyticum were detected in the placentas of 10 (63%) of the infants who died and 26 (27%) of the 96 surviving preterm infants (rate ratio 3.8; 95% confidence interval 1.4 to 11.6). After adjustment for gestational age at delivery, the relationship lost statistical significance. Death occurred in 5 (45%) of 11 preterm neonates with E. coli or group B Streptococcus isolated from the chorioamnion. Of the 16 infants who died, 7 with birth weights <800 gm were stillborn, 3

^{*}U. urealyticum excluded.

Table II. Microorganisms recovered from chorioamnion cultures

	Weeks' gestation at delivery					
	<u> </u>	34	>	·34		
,	No.	%	No.	%	p value	
Genital mycoplasmas						
Ureaplasma urealyticum	24	21	30	19	0.8	
Mycoplasma hominis	2	2	1	0.6	0.6	
Facultative bacteria						
Group B Streptococcus	8	7	3	2	0.05	
Escherichia coli	5	4	1	0.6	0.08	
Gardnerella vaginalis	6	5	15	10	0.3	
Viridans streptococci	8	7	8	5	0.7	
Enterococcus	6	5	6	4	0.8	
Lactobacillus	2	2	2	1	0.99	
Anaerobic bacteria						
Peptostreptococcus*	12	11	4	3	0.006	
Fusobacterium	4	4	0	0	0.03	
Bacteroides†	5	4	1	0.6	0.08	
Actinomyces	2	2	1	0.6	0.6	
Mobiluncus	1	1	0	0	0.4	
Yeast						
Candida albicans	1	1.	0 `	0	0.41	

^{*}Peptostreptococcus species include P. asaccharolyticus (11 cases), P. magnus (3), P. anaerobius (2).

Table III. Association of bacterial isolates from the chorioamnion with histologic chorioamnionitis

	Chorioan		
	No.	- %	p value*
Genital mycoplasmas			
Ureaplasma urealyticum	29/54	54	0.002
Mycoplasma hominis	2/3	66	0.2
Facultative bacteria			
Group B Streptococcus	7/Ì1	64	0.04
Escherichia coli	5/6	83	0.01
Gardnerella vaginalis	9/21	43	0.3
Viridans streptococci	8/16	50	0.2
Enterococcus	4/12	33	0.8
Lactobacillus	2/4	50	0.6
Anaerobic bacteria			
Peptostreptococcus	7/16	44	0.3
Fusobacterium	4/4	100	0.009
Bacteroides	5/6	83	0.01
Actinomyces	0/3	0	0.6
Sterile	52/176	30	Reference

^{*}Compared with the sterile culture group, Fisher's exact test (two-tailed).

with birth weights <1200 gm died within the first day of life, and 6 with birth weights <1200 gm had suspected sepsis and died of respiratory complications commonly associated with prematurity.

Respiratory distress syndrome developed among 43 (45%) of the 96 surviving neonates. Respiratory distress syndrome was weakly associated with chorioamnion infection by *Ureaplasma* or other bacteria and with histologic chorioamnionitis (Table V). After adjustment for gestational age at delivery, this association lost statistical significance.

Comment

The overlying hypothesis for this study was that upper genital tract infection represents one cause of preterm birth. Chorioamnion infection was related to preterm birth in this study after adjustments were made for duration of membrane rupture and labor and other demographic and behaviorial variables. The isolation of specific microorganisms from the chorioamnion was associated with delivery at ≤34 weeks' gestation and with histologic chorioamnionitis. Group B streptococci were associated with both delivery at ≤34 weeks' ges-

[†]Bacteroides species include B. bivius (2 cases), B. ureolyticus (1), B. asaccharolyticus (1), B. fragilis (1), and Bacteroides sp. (1).

Table IV. Relationship of neonatal death to indicators of chorioamnion infection among women who were delivered before term

	Neonate	al death	U1	nadjusted	Ad	justed†
Chorioamnion	% Yes $(n = 16)$	% No. $(n = 96)$	Rate ratio*	95% CI	Rate ratio*	. 95% CI
<i>Ureaplasma</i> Other bacteria Inflammation	27 63 63	21 27 58	1.3 3.8 1.2	0.4-4.1 1.4-11.6 0.4-3.2	0.7 2.0 0.7	0.2-2.3 0.7-5.7 0.2-2.0

CI, Confidence interval.

Table V. Relationship of respiratory distress syndrome to indicators of chorioamnion infection in surviving neonates delivered preterm

	Respiratory di	Respiratory distess syndrome		adjusted	Adjusted*	
Chorioamnion	% Yes $(n=43)$		Rate ratio	95% CI	Rate ratio	95% CI
<i>Ureaplasma</i> Other bacteria Inflammation	31 37 70	14 19 49	1.7 1.6 1.7	1.1-2.6 1.0-2.4 1.0-2.7	1.2 1.3 1.3	0.9-1.7 0.9-1.8 0.9-2.0

Neonatal deaths excluded. CI, Confidence interval.

tation and chorioamnionitis, and *E. coli* was significantly associated with histologic chorioamnionitis. These two microorganisms are the leading causes of neonatal sepsis and meningitis. ¹⁹ Our findings suggest that group B streptococci and *E. coli* are emerging as important placental pathogens.

Fusobacterium species have rarely been recovered from the chorioamnion in previous studies, although this organism is an important pathogen in the amniotic fluid of women in preterm labor who have intact membranes.11 Amniotic fluid Fusobacterium and Bacteroides bivius have also been significantly associated with delivery of infants weighing <2500 gm among women with intraamniotic infection.20 In our study Fusobacterium and Peptostreptococcus in the chorioamnion were associated with delivery at ≤34 weeks' gestation, and Fusobacterium and Bacteroides in the chorioamnion were associated with histologic chorioamnionitis. Anaerobes are infrequent isolates from neonates with suspected sepsis,19 although exceptions exist.21 Infant death among neonates with anaerobic infections of the chorioamnion could be related to preterm delivery or unrecognized neonatal infection with these bacteria, or it could be an indirect effect of toxins, inflammation, or suppressed leukocyte function.

U. urealyticum has long been recognized as a placental pathogen. Recovery of U. urealyticum from the cho-

rioamnion and newborn infant has been significantly related to histologic chorioamnionitis among term-delivered infants. U. urealyticum placental infection has been associated with prematurity, low birth weight, intrauterine growth retardation, and chorioamnionitis among preterm infants. Salation of U. urealyticum from the lower respiratory tract has been associated with chronic lung disease and death in very low birth weight infants. In our study, the recovery of U. urealyticum from the chorioamnion was related to histologic chorioamnionitis but not to delivery at ≤ 34 weeks' gestation. Our data suggest that infants exposed to U. urealyticum chorioamnion infection do not have increased mortality rates.

We defined prematurity as a gestational age of ≤ 34 weeks because evidence of chorioamnion infection and histologic chorioamnionitis is highest in the group delivered at ≤ 34 weeks' gestation. The prevalence of chorioamnion infection and histologic chorioamnionitis. is stable at > 35 weeks' gestation. Further, most of the morbidity and mortality related to prematurity occurs among infants born at ≤ 34 weeks' gestation. The relationship of chorioamnion infection and neonatal death could operate through the mechanism of infection causing prematurity. If this mechanism were established with certainty, adjustment for gestational age would not be appropriate. Never-

^{*}Time to event: number of days between birth and death.

[†]Adjusted for gestational age at delivery.

^{*}Adjusted for gestational age at delivery by Mantel-Haenszel stratified analysis.

theless, we elected to adjust for gestational age to examine whether chorioamnion infection per se was related to neonatal death independent of gestational age. Larger studies of placentas from preterm deliveries are needed to determine whether chorioamnion infection further increases the high neonatal death rate from prematurity in infants born at low gestational ages.

Chorioamnion infection is statistically related to histologic chorioamnionitis in this and several other studies. 1-3, 7, 8 However, some microorganisms in the placenta (U. urealyticum, group B Streptococcus, E. coli, Fusobacterium, and Bacteroides) appear to produce inflammation and others do not. The association between chorioamnion bacteria and prematurity is complex and does not simply relate to histologic inflammation: Peptostreptococcus in the chorioamnion was highly related to prematurity but not to histologic chorioamnionitis. U. urealyticum was related to histologic inflammation but not to delivery at ≤34 weeks' gestation. Further, only one third of chorioamnions with inflammation yielded microorganisms, and only 58% of infected chorioamnions had inflammation. Our data support the concept that histologic chorioamnionitis and infection are related but distinct entities.

It is not possible to demonstrate a cause-and-effect relationship between infection and preterm birth by using this study design; however, the data in this report suggest that only certain microorganisms in the chorioamnion are associated with preterm delivery. It is possible that certain bacteria primarily invade the chorioamnion, elicit an inflammatory response, and initiate labor leading to preterm delivery and that other microorganisms secondarily invade the chorioamnion during labor and are equally frequent among preterm and term women and in the chorioamnions with and without inflammation. Alternatively, it is possible that some microorganisms not independently associated with preterm birth play a role in preterm birth when present in combination with other bacteria. Larger studies will allow more detailed examination of the relationship between individual bacterial isolates and prematurity after adjusting for length of labor or membrane rupture.

Our data suggest that *U. urealyticum*, group B Streptococcus, E. coli, Bacteroides, Fusobacterium, and Peptostreptococcus should be considered as important placental pathogens associated with delivery at ≤34 weeks' gestation and histologic chorioamnionitis. Clinically useful methods to diagnose chorioamnion infections before delivery are not yet available. When these methods are developed, antibiotic therapy can be evaluated for efficacy in prolonging pregnancy among those women in preterm labor who have infections. The prevention of preterm delivery and neonatal sequelae by directed antibiotic therapy would support a primary rather than a secondary role of infection in preterm delivery.

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Incidence and prevalence of human immunodeficiency virus infection in a prenatal population undergoing routine voluntary human immunodeficiency virus screening, July 1987 to June 1990

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To characterize the epidemiologic characteristics of human immunodeficiency virus type 1 infection in an urban prenatal population in the southeastern United States, we conducted serial routine voluntary antenatal human immunodeficiency virus antibody testing and obtained self-reported human immunodeficiency virus risk behavior profiles on women registering for prenatal care. From July 1987 to June 1990, 23,432 women registered for prenatal care. The majority of women (95%) consented to human immunodeficiency virus antibody testing and completed risk behavior profiles. The cumulative incidence of human immunodeficiency virus infection increased from 3.5 per 1000 in 1987 and 1988 to 5.3 per 1000 in 1989 and 1990. A history of "crack" cocaine use emerged as a significant risk factor for infection (p < 0.01). The majority (70%) of human immunodeficiency virus—infected women did not self-acknowledge risk factors for infection and would not have been identified if screening had been targeted. The increasing incidence of human immunodeficiency virus type 1 infection in our prenatal population reinforces the need for our continued routine voluntary antenatal human immunodeficiency virus screening and risk behavior assessment. (AM J OBSTET GYNECOL 1991;165:961-4.)

Key words: Incidence of human immunodeficiency virus infection, voluntary antenatal antibody screening, "crack" cocaine

From 1988 to 1989 the Centers for Disease Control (CDC) reported a 29% increase in cases of acquired immunodeficiency syndrome among women. In 1990 women represent the group with the fastest rate of increase in human immunodeficiency virus (HIV) infection and will account for 11% of cases of acquired immunodeficiency syndrome in the United States. The consequences of increasing HIV infection could potentially be devastating, for women, for their sex partners, and for their infants. For their sex partners, and for their infants.

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The incidence of HIV infection in pregnancy and risk behaviors for infection have been poorly characterized. Most published studies to date have been blinded surveys that failed to link serologic prevalence with risk behaviors. Find This report describes the results of 3 years of routine voluntary antenatal HIV antibody screening and linked risk behaviors. The objectives of this study are twofold: to monitor the incidence of HIV infection in women who receive prenatal care at our institution and to characterize the risk behavior for infection. Because of our policy of routine voluntary HIV screening we have the unique opportunity to conduct this epidemiologic survey in a large, well-characterized subset of women of reproductive age.

Material and methods

Patients. Between July 1, 1987, and June 30, 1990, a study of HIV incidence in prenatal patients was con-

Table I. Selected demographic characteristics of seropositive and seronegative pregnant women undergoing routine voluntary HIV screening, Grady Memorial Hospital, July 1987–June 1990

	Seropositive $(n=113)$		Seroneg $(n = 22,$		
Characteristics	No.	%	No.	%	p Value
Age (yr)					
<16	2 ·	1.8	1,107	5.2	
16-20	31	27.4	7,671	36.2	
21-25	46	40.7	6,689	31.6	
26-30	19	16.8	3,613	17.0	0.04
31-35	14	12.4	1,575	7.4	
≥36	I	0.9	530	2.5	
Race					
Black	98	86.7	17.542	82.9	
White	11	9.7	2,473	11.7	NS
Other .	4	3.5	1,138	5.4	
Marital status					
Single	85	75.2 ·	14,390	69.5	
Married '	15	13.3	4,038	19.5	NS
Divorced/separated	13	11.5	2,271	10.9	
Years of schooling					
<12	37	32.7	8,292	37.9	
12	57	50.4	9,175	41.9	ŅS
>12	19	16.8	4,418	20.2	,

NS, Not significant.

*Percentages are based on the actual number of cases found for each category. Because records for pregnant women were missing or contained incomplete information, categories may not total 100%.

ducted at Grady Memorial Hospital. Grady Memorial Hospital is a 1000-bed county hospital in Atlanta, which provides care for a predominantly black inner-city population. During the study period there were 23,587 deliveries and 4500 abortions.

The framework of routine voluntary antepartum HIV screening in 1987 and 1988 has been described in a previous report.¹⁰ In 1988 and 1989 each woman received information prepared by the Georgia Department of Human Resources on acquired immunodeficiency syndrome and HIV testing11 before granting informed consent. In 1989 and 1990 the pretest counseling material was supplemented by 30 minutes of HIV counseling. Patients were counseled in small groups of 5 to 7. All counseling sessions were conducted by trained HIV counselors. Before counseling all women were asked to complete a self-administered questionnaire, which assessed risk factors for infection. In addition, after being informed of infection, all women with seropositive results were asked to complete an in-depth questionnaire assessing risk factors for infection.

Laboratory. Specimens were screened for HIV-1 antibody with a commercial solid-phase enzyme immunoassay (Abbot HTLV-III EIA; Abbot Laboratories, North Chicago) according to the manufacturer's assay protocol. Specimens that were repeatedly reactive by this assay were examined by Western blot technique (Biotech DuPont HTLV-III Western blot kit, DuPont Pharmaceuticals, Wilmington, Del.). Specimens with

reactivity to any antigen band in two of three viral gene product groups were considered HIV-1 seropositive.

Data analyses. Clinical and demographic data were recorded onto computer tape and machine edited. The estimated incidence rate is expressed as the number of HIV-1—positive specimens divided by the total number tested for that year. Seropositive women identified prior to pregnancy were not included in incidence calculations. Statistical analysis included χ^2 test, which measured differences between proportions. Significance is expressed at the 0.05 level.

Results

During the 3-year study period 23,432 pregnant women registered for prenatal care; 22,364 (95.4%) consented to HIV antibody testing and completed the self-administered HIV risk behavior questionnaire. Of the 22,364 women screened for HIV infection 113 (5.0/1000) had seropositive results by Western blotsupplemented testing. The yearly cumulative incidence of HIV infection increased from 3.5 per 1000 in 1987 and 1988 to 5.3 per 1000 in 1989 and 1990, which was not significant. Three women were known to be HIV infected before pregnancy. All women but one had asymptomatic HIV infection. The racial, marital, and educational status of seropositive and seronegative women was similar. However, the age distribution was significantly different (p < 0.05) (Table I). This is accounted for primarily by the larger percentage of seropositive results in women in the 21- to 25-year age

Table II. Comparison of self-reported HIV risk factors in pregnant women undergoing routine voluntary antibody screening, Grady Memorial Hospital, July 1987-June 1990

	1987- (n =	-1988 7356)	1988- (n = 1		1	-1990 7604)	
Risk factors	No.	%	No.	%	. No.	%	p Value
Intravenous drug use	109	1.5	82	1.1	136	1.8	NS
"Crack" cocaine use	57	0.8	92	1.2	393	5.2	< 0.01
Transfusion since 1978	24	0.3	177	2.6	236	3.1	< 0.01
Partner has AIDS or ARC	23	. 0.3	17	0.3	43	0.57	< 0.01

NS, Not significant, AIDS, acquired immunodeficiency syndrome; ARC, acquired immunodeficiency syndrome-related complex.

Table III. Comparison of risk factors for HIV infection obtained from in-depth questionnaire in pregnant women with seropositive results undergoing routine voluntary antibody screening, Grady Memorial Hospital, July 1987-June 1990

	I .	'-1988 = 26)	1988- (n =			9-1990 = 40)	
Risk factor*	No.	%	No.	%	No.	%	p Value
Undetermined (no risk factor)	13	50	21	45	16	40	NS
Heterosexual contact	8	30.6	16	34	7	17.5	NS
Intravenous drug use	3	11.5	10	21	7	17.5	NS
"Crack" cocaine use	1	3.8	0	0	9	21.4	< 0.01
Transfusion	1.	3.8	0	0	1	2.5	NS

NS, Not significant.

group. There was a significant increase in self-reported HIV risk behavior from 9.4% in 1987 and 1988 to 15.2% in 1989 and 1990 (p < 0.05). The increase in self-reported risk behavior is attributable in part to significant increases in HIV-infected sexual partners and use of "crack" cocaine (p < 0.01) (Table II). During the 3-year study interval, risk factors for HIV infection among women with seropositive results were nearly comparable except for a significant increase in "crack" cocaine use (p < 0.01) (Table III). Before HIV antibody testing, 34 (29.1%) women with seropositive results acknowledged risk factors for infection. After being informed of their serologic status, an additional 29 (25.7%) women acknowledged risk factors for infection.

During the 3-year study interval, 10 of 57 (17.5%) women with seropositive results who were eligible for abortion by gestational age criteria terminated their pregnancies.

Comment

Incidence data on HIV infection in women of reproductive age are extremely important. These data may assist public health officials in projecting health expenditures for HIV-related care and planning prevention strategies. During the 3-year study period we found a 1.5-fold increase in incidence of HIV infection among women registering for prenatal care. The increasing incidence likely represents increasing infection in pregnant women in our community.

The prevalence of HIV infection of 5.8 per 1000 in childbearing women seen at Grady Memorial Hospital in 1988 to 1990 was 3.6-fold higher than the 1.6 per 1000 childbearing women reported from statewide heel stick data from Georgia.9 In addition, the cumulative incidence of HIV infection of 5 per 1000 pregnancies is 3.5-fold higher than the CDC's 1989 national seroprevalence estimates that are based on cord blood testing of 1.5 per 1000 childbearing women.9 On the basis of the current annual incidence of HIV infection in our institution and assuming either a decreasing or stable rate of infection, we estimate that 35 to 40 pregnant women per year who register for prenatal care will be HIV infected. However, it is possible that the incidence of HIV infection will continue to increase, yielding >40 HIV-infected pregnant women per year.

Risk behaviors in HIV-infected parturients provide important insights about potential mechanisms of spread of infection. A disturbing finding was the approximate doubling of self-reported HIV risk behavior over time. The increase in risk behavior closely parallels the increase in incidence. From our analysis there ap-

^{*}Risk factors are assumed to be mutually exclusive.

pears to be an emerging relationship between increase "crack" cocaine use and HIV infection. During the 3year study interval we observed a sixfold increase in self-reported "crack" cocaine use among all parturients. In addition, we found a fivefold increase in "crack" cocaine use as a potential risk factor for infection among HIV-infected pregnant women. "Crack" cocaine use is believed to increase sexual risk taking and is likely to increase HIV risk by this mechanism.

In this study self-selection bias was minimized, as 95% of parturients eligible for screening consented to antibody testing and completed risk behavior questionnaires. In this as in other studies6, 10, 12 there was a poor correlation between targeted screening and HIV infection. Nearly three fourths of seropositive pregnant women had no self-identified risk factors for infection and would not have been identified if our policy of routine voluntary antibody screening had not been in place. Our 3-year experience has shown us that routine voluntary antenatal HIV screening is a valuable component of comprehensive prenatal care. We believe that such testing must be offered on a voluntary basis, with written informed consent with ensured confidentiality,10 and without impeded access to prenatal care. Through such screening 112 HIV-infected women without symptoms were detected during the study interval. These women were educated about the major modes of virus transmission, encouraged to practice risk-reduction behavior, provided contraceptives, and referred for both pediatric and adult infectious disease follow-up. The identification of HIV-infected women without symptoms has immediate urgency in light of the recent clinical trials that reveal benefits of low-dose zidovudine in asymptomatic HIV infection.13

In spite of the known risk of perinatal transmission of HIV infection, 84% of infected women eligible to have elective abortions declined the procedure. Therefore in our institution termination of pregnancy in HIV-infected women will be chosen infrequently as a means to prevent perinatal transmission.

These data represent the first report of the estimated incidence of HIV infection in a large, well-characterized prenatal population. Our findings are a direct indication of the growth of the epidemic in our community.

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Natural killer cell cytotoxicity to herpes simplex virus-1-infected cells is not altered by pregnancy

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There is evidence to suggest a decrease in natural killer cell cytotoxicity during pregnancy, but information regarding immune responsiveness to actual infection is limited. An in vitro study was undertaken to examine the effect of herpes simplex virus infection on natural killer cell cytotoxicity with peripheral blood mononuclear cells from pregnant (N=8) and nonpregnant (N=5) women. The peripheral blood mononuclear cells were separated by Ficoll-Hypaque centrifugation. Effector cells were incubated with live herpes simplex virus-1, ultraviolet-inactivated herpes simplex virus-1, or media alone for 18 hours at 37° C. K562 target cells were used in a sodium chromate release assay with an effector-to-target cell ratio of 100:1. Baseline natural killer cell values (mean \pm SE) for pregnant patients (13.4% \pm 2.4%) and nonpregnant patients (19.8% \pm 3.7%) were similar. Natural killer cell cytotoxicity was significantly increased by incubation with live virus for both pregnant (37.5% \pm 6.2%) and nonpregnant subjects (49.8% \pm 7.6%). There was no difference in mean values between media and ultraviolet-inactivated herpes simplex virus-1-exposed samples for either group. Results suggest that (1) infection with live virus, but not viral antigen alone, can augment natural killer cell response in vitro and (2) natural killer cell response to herpes simplex virus-1 infection is not altered by pregnancy. (AM J OBSTET GYNECOL 1991;165:965-8.)

Key words: Natural killer cell cytotoxicity, herpes simplex virus, pregnancy

Natural killer cells are distinct from cytotoxic T lymphocytes in their ability to lyse target cells without prior antigenic sensitization. Therefore they play a primary role in the immune defense system against viral infection, including herpes simplex virus (HSV).

Several reports suggest that pregnant women have a higher incidence of HSV infection, with a longer duration and severity of clinical symptoms.¹⁻⁴ However, epidemiologic data to support this suggestion are lacking.⁵ Several authors have shown a decrease in natural killer cell cytotoxicity in pregnant subjects compared with nonpregnant controls.⁶⁻¹² This has led some investigators to refer to pregnancy as an immunocompromised state. There is limited information regarding natural killer cell immune responsiveness to actual infections in humans.

An in vitro study was undertaken to examine the effect of HSV infection on natural killer cell cytotoxicity with peripheral blood mononuclear cells from pregnant and nonpregnant subjects. We hypothesized that natural killer cell cytotoxicity would be augmented by HSV infection in a similar fashion for both groups.

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Material and methods

Patients. After written informed consent was obtained, 20 ml of blood was obtained into heparinized tubes from eight pregnant subjects and five reproductive-aged nonpregnant controls.

Preparation of effector cells. Dextran (3%, Sigma Chemical Co., St. Louis) was added to each tube (10%, vol/vol) and allowed to sediment for 45 minutes. The plasma was then placed over 10 ml of Ficoll-Hypaque (Pharmacia Laboratories, Inc., Piscataway, N.J.) and centrifuged at 200 g for 45 minutes. The mononuclear cell layer was washed three times in Hank's balanced salt solution (Hazelton, Lenexa, Kan.) and resuspended in 1 ml minimal essential media (Gibco Live Technologies, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (Hazelton), penicillin (50 U/ml), and streptomycin (50 μg/ml). These mononuclear cells are a heterogeneous cell population consisting of lymphocytes, macrophages, and natural killer cells.

Target cells. K562 (an erythroleukemia cell line) target cells were grown in culture. A pellet was obtained by centrifuging 10 ml at 200 g for 5 minutes. The pellet was resuspended in 150 μ l of 10% minimal essential media and incubated with 20 to 80 μ l sodium chromate (Dupont, Wilmington, Del.) for 1 hour at 37° C. The cells were then layered over 3 ml heat-inactivated fetal calf serum and centrifuged for 5 minutes at 200 g. The pellet was then washed with 10 ml 10% minimal essential media. The supernatant was discarded, 1 ml of 10%

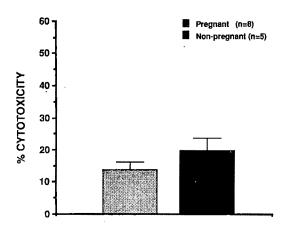


Fig. 1. Baseline natural killer cell cytotoxicity (mean \pm SE) in pregnant and nonpregnant subjects (p=0.25). Effector/target cell ratio equals 100:1.

minimal essential media was used to resuspend the pellet, and the cells were counted. The sodium chromate–labeled target cells were suspended at a final concentration of 1×10^5 cells per milliliter in 10% minimal essential media.

Microcytotoxicity assay. Each assay was conducted in triplicate in polystyrene U-bottom-well microtiter plates (Corning, Corning, N.Y.). The unstimulated wells, for pregnant and nonpregnant subjects, consisted of 50 µl effector cells and 50 µl 10% minimal essential media, yielding a final concentration of 5×10^6 cell per milliliter. Each of the HSV-stimulated wells consisted of 50 µl effector cells and 50 µl either live HSV-1 virus (2 × 10⁵ plaque-forming units) or 50 μl ultravioletinactivated HSV-1 (2 × 10⁵ plaque-forming units). The final concentration of effectors was 5 × 106 cells per milliliter with 1×10^5 plaque-forming units of HSV. The cells were incubated for 18 hours at 37° C. Experimental wells that contained live virus were washed, along with the unstimulated wells, three times with 10% minimal essential media. Subsequently, 50 µl of 10% minimal essential media and 50 µl of labeled target cells $(1 \times 10^{5} \text{ cells per milliliter})$ were added to all of the wells. The final effector-to-target cell ratio was 100:1. The covered plate was then centrifuged at 75 g for 5 minutes and allowed to incubate an additional 4 hours at 37° C in a humidified atmosphere of air (95%) and carbon dioxide (5%). Afterward 100 µl was aspirated from the top of each well without disturbing the pellet. To each well, 100 µl of 1 mol/L sodium hydroxide was added, and the total volume was aspirated into a separate tube. The samples were counted in a Packard Cobra 5010 y-counter for 1 minute. Chromium release was calculated by the formula:

$$\frac{\text{Percent sodium}}{\text{chromate release}} = \frac{2A}{A + B} \times 100$$

where A equals counts per minute in the top 100 μ l

and *B* equals counts per minute of the lysed pellet.

Natural killer cell cytotoxicity equals:

$$\frac{\text{Experimental release} - \text{Spontaneous release}}{100 - \text{Spontaneous release}} \times 100$$

Data are expressed as the mean \pm SE of experiments. These data were analyzed with the arcsine transformation. Comparisons were made between the two groups by the analysis of variance, with p < 0.05 considered statistically significant.

Results

Baseline natural killer cell cytotoxicity (mean \pm SE) was lower in pregnant subjects (13.8% \pm 2.4%) than in nonpregnant subjects (19.8% \pm 3.7%) (Fig. 1). However, this did not reach statistical significance (p=0.25). Incubation of effector cells with live HSV-1 significantly increased natural killer cell cytotoxicity for pregnant (37.5% \pm 6.2%) and nonpregnant (49.8% \pm 7.6%) subjects, when compared with values for media alone (Fig. 2). As anticipated, the increase in cytotoxicity was similar for both groups (p=0.25) (Fig. 2).

Similar experiments with ultraviolet-inactivated HSV-1 (1×10^5 plaque forming units) showed no difference in mean natural killer cell cytotoxicity values (Fig. 3) when compared with those for media alone for either group. Additional experiments were conducted with 1×10^7 plaque forming units of ultraviolet-inactivated HSV-1, to determine if increasing the amount of antigen would have any effect on cytotoxicity. Again, no difference in mean natural killer cell cytotoxicity values were noted for either group (results not depicted).

Comment

Pregnancy represents a unique immunologic state in which a balance exists between acceptance of the fetal allograft and host immunocompetence. A suppression in maternal cell-mediated immunity is assumed to be advantageous for the fetus but theoretically makes the pregnant woman more vulnerable to infections, particularly those of viral causes. Case reports^{3, 4} and animal work¹³ suggest that the severity of an HSV infection is increased in pregnancy. A higher incidence of HSV infections and a longer duration of clinical symptoms have been reported in pregnancy. Although firm epidemiologic data are lacking to confirm these observations,⁵ these clinical reports have led to the suggestion that pregnancy is an immunocompromised state.

Natural killer cells represent the first line of defense against viral pathogens and therefore play an important role in HSV infection. Data in regard to natural killer cells in pregnancy are limited and sometimes conflicting. Histochemical studies⁹ and flow cytometry¹⁰

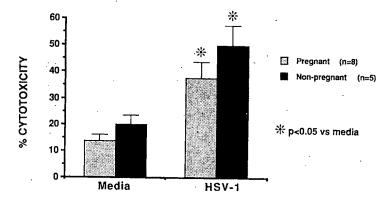


Fig. 2. Natural killer cell cytotoxicity in response to live HSV-1 infection. Results (mean \pm SE) show pregnant and nonpregnant subjects compared with controls (media alone, p < 0.05). Effector/target cell ratio is 100:1.

have shown that the number of natural killer cells is not reduced during pregnancy. Natural killer cell function has been examined in several recent studies, 7-12 which demonstrate a general decrease in natural killer cell activity during pregnancy, although this finding is not consistent. None of these previous studies have attempted to examine the effect of actual infection on natural killer cell immune responsiveness.

To address this question, an in vitro model was developed in which peripheral blood mononuclear cells: of pregnant and nonpregnant subjects were infectedwith live HSV-1 virus. Natural killer cell cytotoxicity was measured in control and infected wells to determine if pregnancy altered immune responsiveness to infection. Our results in Fig. 1 show that baseline cytotoxicity tended to be lower in pregnant subjects, compared with nonpregnant controls. Although this did not reach statistical significance, the calculated power of these experiments (0.54) to reveal a significant reduction in natural killer cells (20%) is limited. To have a power = 0.8, 14 paired experiments would need to be analyzed. Regardless, our results are consistent with data already available in the literature7-12 and are not central to the issue of HSV-induced immune responsiveness. Incubation of effector cells with live HSV-1 resulted in a significant increase in natural killer cellcytotoxicity as compared with controls for both groups (Fig. 2). There was a 2.5-fold increase in cytotoxicity in the nonpregnant subjects, compared with a 2.7-fold increase in the pregnant subjects. This demonstrates that, in spite of lower baseline natural killer cell cytotoxicity values in the pregnant subjects, they were able to respond in a similar fashion as the nonpregnant subjects (Fig. 2).

Viral infection leads to the production of a variety of cytokines. Several of these cytokines, in particular interferon alfa, are known to regulate natural killer cell function. Deficiencies in cytotoxic responses have been shown to result in a greater susceptibility to HSV in-

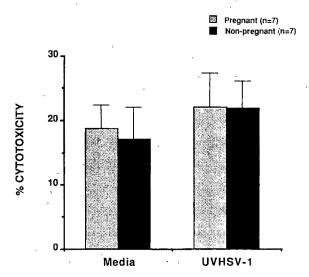


Fig. 3. Natural killer cell cytotoxicity in response to ultraviolet-inactivated HSV-1. Effector/target cell ratio is 100:1.

fection.^{14, 15} Furthermore, natural killer cell activity aginst HSV infection is enhanced by interferon in vitro, ^{16, 17} and in vivo. ¹⁸ Data from our laboratory confirm that interferon alfa can augment natural killer cell cytotoxicity in the pregnant host. ¹⁹

One could speculate that, although baseline natural killer cell activity during pregnancy may be decreased, cytokine production in response to infection may be normal, accounting for our results. Evidence to support this includes the ability of interferon to restore natural killer cell activity from peripheral blood of pregnant patients, 10 and systemic interferon production is higher in pregnant mice than in controls. 13 Surprisingly, few data regarding cytokine production in human pregnancy exist. Studies examining the production of interleukin-120 and interleukin-221 demonstrate elevated or normal production rates, respectively, compared

with those of nonpregnant controls. We have shown that the monokines tumor necrosis factor and interleukin-6 also are significantly increased during pregnancy (unpublished observations). Although interferon alfa has not been directly measured during pregnancy, these data support the concept that its production during pregnancy is unchanged.

Interestingly, we found that ultraviolet-inactivated HSV-1 did not augment natural killer cell activity as did infection with live virus (Fig. 3). This suggests that infection with live virus may be necessary to induce the intracellular production of interferon alfa. Kirchner et al.22 demonstrated that interferon alfa could be detected after injection of live herpesvirus in mice but not with ultraviolet-inactivated herpes antigen. However, HSV antigen in much higher concentrations, and with extended incubation, has been shown to induce lymphocyte stimulation in previously sensitized cells.^{23, 24} The mechanism for this induction has not been identified, but it is most likely humoral in origin. Data from lymphocyte stimulation experiments cannot be extrapolated to explain natural killer cell function, because natural killer cells lyse target cells without prior antigenic sensitization.

In conclusion, our results suggest that (1) infection with live virus, but not viral antigen, can augment natural killer cell cytotoxicity in vitro and (2) natural killer cell response to HSV-1 infection is not altered by pregnancy. We speculate that, during pregnancy, the production of interferon alfa is not attenuated in response to infection with live herpesvirus. This could explain the increase in natural killer cell cytotoxicity in vitro observed in both pregnant and nonpregnant subjects. Although our data suggest that isolated mononuclear cells respond appropriately to HSV infection during pregnancy, other factors, such as serum-mediated suppression of immune activity, may alter in vivo host responses.

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Systemic administration of interleukin-1 induces preterm parturition in mice

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Interleukin-1 has been postulated as a signal for the initiation of preterm labor and delivery. Interleukin-1 is produced by human decidua, stimulates prostaglandin production by intrauterine tissues, and is present in the amniotic fluid of women with preterm labor and intraamniotic infection. The purpose of these studies was to determine whether interleukin-1 could induce parturition in an animal species. Timed-pregnant C3H/HeJ inbred mice (n=24) (genetically endotoxin resistant) were randomized to receive either recombinant human interleukin-1 or sterile phosphate-buffered saline solution between days 15 and 17 of gestation (normal length of pregnancy, 20 to 21 days). Three consecutive subcutaneous injections of interleukin-1 or phosphate-buffered saline solution were administered within 6 hours. Examinations of the animals were performed by blinded observers. Parturition occurred within 24 hours in all of the interleukin-1—treated mice and in none of the control group. Vaginal bleeding was first noted 4 hours after the first interleukin-1 injection, and delivery began within 12 hours after the last interleukin-1 injection. Premature delivery occurred in all interleukin-1—injected mice. Laparotomy revealed that there were no remaining fetuses in utero. All mice in the control group delivered spontaneously between days 20 and 22. We conclude that systemic administration of interleukin-1 induces preterm labor and delivery in mice. (AM J OBSTET GYNECOL 1991;165:969-71.)

Key words: Interleukin-1, cytokines, parturition, labor, chorioamnionitis, prematurity, preterm labor, mice

There is a strong association between systemic or intrauterine infection and preterm labor and delivery (reviewed in reference 1). It has been estimated that at least 20% of preterm births occur in women with microbial invasion of the amniotic cavity.2 However, the mechanisms responsible for the initiation of parturition in the setting of infection have not been elucidated. Interleukin-1 (IL-1), a cytokine produced by human decidua in response to bacterial products,8 has been proposed as a signal for the initiation of labor in the setting of infection.4 Although IL-1 stimulates prostaglandin production by human amnion⁵ and decidua⁶ and is present in the amniotic fluid of women with preterm labor and microbial invasion of the amniotic cavity, there is no direct evidence that this cytokine can induce parturition.4 The purpose of these studies was to determine whether systemic administration of IL-1 can induce parturition in mice.

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Material and methods

Animals. Mice were selected as the experimental animal because of the investigators' familiarity with murine reproductive biology and the considerable information available regarding the immune system and cytokine physiology in this species. Studies were conducted with 19-week-old C3H/HeJ mice that were impregnated by C57BL/6J X DBA/2 F-1 male mice. Animals were allowed free access to food and water before and during experimentation and were exposed to a 12-hour-light/12-hour-dark cycle. Under these conditions mating occurs during the mid—dark period. Mating date, as evidenced by vaginal plug, was designated day 1 of pregnancy.

Experimental design. Twenty-four mice were randomized to receive human recombinant IL-1 α (Hazelton Laboratories, Baltimore) (n=12) or sterile phosphate-buffered saline solution (n=12) subcutaneously. Gestational ages were 15 days (n=12), 16 days (n=4), and 17 days (n=8). Normal duration of pregnancy in this mating combination is 20 to 21 days. Randomization was stratified for gestational age.

Injections consisted of 1 μ g of IL-1 α diluted in 100 μ l of sterile phosphate-buffered saline solution or 100 μ l of phosphate-buffered saline solution. The endotoxin content of this preparation of IL-1 α is <0.1 ng/mg protein (determined with the limulus amebocyte lysate assay). IL-1 α , rather than IL-1 β , was selected for these experiments because IL-1 α is the main IL-1 spe-

1 /	Time							
Gestational age	4:40 PM	5:20 РМ	7 PM	7:45 PM	12 Midnight			
Day 15 $(n = 6)$	1 .	3	4	5	5*			
Day 16 $(n = 2)$	2	2	2	2	2			
Day 17 (n = 4)	0	0	0	3	3			

Table I. Frequency of vaginal bleeding according to gestational age in animals receiving IL-1

cies produced by murine macrophages. The dose of IL- 1α selected for these experiments has been shown to be optimal for the induction of an acute-phase response in mice.⁷ Injections were performed at 12 noon, 2:15 PM, and 5:30 PM. The investigators observing the animals were blinded to the treatment protocol.

Results

Behavioral changes (piloerection and lethargy) were noted at the time of the second injection in all IL- 1α treated animals and in none of the control animals. At the time of the third injection one mouse (15 days' gestation) injected with IL-1α became prostrate. Six hours later this mouse was found dead. Vaginal bleeding was first noted 2 hours after the second injection in animals injected with IL-1a. Table I describes the occurrence of vaginal bleeding over time in the IL-1αtreated group. No animals receiving phosphate-buffered saline solution had vaginal bleeding.

Preterm delivery occurred in all 11 IL-1α-treated animals but in none of the phosphate-buffered saline solution-treated group. Within 12 hours of the last injection all IL-1-treated mice were in the process of delivery. Within 24 hours after the last injection 41 dead, premature, newborn mice were counted in the cages. However, several mothers were observed eating recently passed fetuses. Hysterotomy was performed 48 hours after the last injection. No fetuses were found in utero.

Deliveries in the control group occurred on day 20 in all female mice but one. A total of 83 newborn mice were counted. Four were stillborn or died shortly after birth. One mouse, injected on day 15 of pregnancy, delivered four newborn mice on day 22 (three were stillborn and one died 3 days later).

Comment

The traditional paradigm invoked to explain the onset of labor in the setting of infection has been that bacterial products directly stimulate prostaglandin biosynthesis.8 Indeed, bacteria are a source of phospholipase A28 and C,9 and bacteria-conditioned media can stimulate prostaglandin production by human amnion. 10-13 Moreover, lipopolysaccharide, a component of the cell wall of gram-negative bacteria, is frequently

present in the amniotic fluid of women with intraamniotic infections14 and is capable of stimulating prostaglandin production by amnion15 and decidua.16 Additionally, the amniotic fluid concentration of endotoxin in women with preterm labor and premature rupture of membranes was higher than in women with premature rupture of membranes without labor.17

In spite of these data, there is evidence that bacterial products alone are not responsible for the onset of preterm labor in the setting of infection. First, one third of women with preterm premature rupture of membranes without labor have microbial invasion of the amniotic cavity,1 suggesting that the mere presence of microorganisms in the amniotic cavity is not sufficient to lead to the onset of labor. Second, it is now apparent that the effects of microbial products on prostaglandin production by intrauterine tissues are highly dose dependent and often inhibitory.18

The recognition of the key role played by cytokines in the pathophysiologic characteristics of fever, endotoxic shock, and other metabolic derangements associated with infection prompted us to propose that the initiation of labor in the setting of infection could be mediated by cytokines. The evidence to support a role for IL-1 includes the following: (1) IL-1 stimulates prostaglandin production by amnion⁵ and decidua⁶; (2) human decidua can produce IL-1 in response to bacterial products3; (3) amniotic fluid IL-1 bioactivity and concentrations are elevated in women with preterm labor and microbial invasion of the amniotic cavity4; (4) in women with preterm premature rupture of membranes and microbial invasion of the amniotic cavity, amniotic fluid IL-1 bioactivity and concentrations are significantly higher in patients with labor than in those without labor,4 indicating that the secretion of IL-1 into the amniotic fluid is associated with parturition rather than infection; (5) in vitro perfusion of human uteri with IL-1 elicits uterine contractility (Bulletti C, Romero R. Unpublished observations).

The results of the experiments described in this communication indicate that systemic administration of IL-1 induces preterm parturition in mice. The results provide strong support for the view that cytokines may play a role in the onset of parturition. We assume that IL-1 binds to IL-1 receptor-bearing cells within the

^{*}One mouse died.

uteroplacental tissues and induces a cascade of biochemical events leading to uterine muscle contraction and active delivery of premature fetuses. Our observations are apparently at variance with those reported by Silen et al. 19 and Parant. 20 Silen et al. reported fetal death and necrosis of the placenta and decidua after administration of a single injection of IL-1α (100 to 500 µg/kg) to 12-day pregnant rats. However, abortion or premature labor did not occur. Similarly, Parant20 reported fetal death (100% of cases) and some preterm deliveries (rate not stated in the report) after a single intravenous injection of 10 µg per mouse to 12-daypregnant mice. The most obvious differences between these reports and ours are the dose and the timing of IL-1 administration.

The current study is relevant to the occurrence of parturition in cases of systemic febrile illnesses in which IL-1 can be detected in the peripheral circulation. However, further studies are required to establish whether intrauterine administration of cytokines can induce parturition. Such an experimental approach would address the issue of whether increased local intrauterine availability of IL-1 can also induce parturition. Although our results indicate that IL-1 can induce parturition in mice, they do not address the issue of whether IL-1 is a requirement for parturition in the setting of infection. Recently, IL-1 receptor antagonists have become available and should be helpful in testing this hypothesis and also in determining whether the effect of IL-1 in the induction of parturition is receptor mediated.

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Prophylactic amnioinfusion as a treatment for oligohydramnios in laboring patients: A prospective, randomized trial

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Prophylactic amnioinfusion was studied in a randomized sample of 305 patients with oligohydramnios in labor. One hundred seventy-five patients underwent amnioinfusion with the remainder serving as controls. Amniotic fluid was titrated to an amniotic fluid index >10.0 cm in the treatment group. Patients receiving amnioinfusion had significantly less operative intervention for fetal distress (p=0.0001) and fewer cesarean sections (p=0.0001). Umbilical artery pH at the time of delivery also was increased (p=0.0001). Rates of amnionitis and endometritis were not significantly different between infused patients and controls, although the length of hospital stay was significantly decreased (p=0.002) in the treatment group. Our data support earlier reports in the literature that amnioinfusion is a useful technique for decreasing intrapartum morbidity for both mother and fetus. (AM J OBSTET GYNECOL 1991;165:972-5.)

Key words: Amnioinfusion, amniotic fluid index, operative intervention, fetal distress

Amnioinfusion is being used for several indications, including prophylactic treatment of oligohydramnios, elimination of significant variable decelerations in labor,1-3 and the prevention of meconium aspiration in laboring patients with thick meconium.4 Initial studies in the literature have been promising, with decreased operative intervention for fetal distress and improved neonatal outcome reported.5 These findings are believed to be due to the restored cushioning effect of amniotic fluid and the subsequent prevention of cord compression. These initial studies, however, have raised questions about the use of amnioinfusion in statistically reducing the incidence of cesarean section for fetal distress and the relationship between amnioinfusion and intrapartum or postpartum infection.5,6 The purpose of this study was to examine the effect of prophylactic amnioinfusion on the incidence of operative intervention for fetal distress, the fetal outcome, and its effect on the incidence of intrapartum and postpartum infection.

Material and methods

Between Aug. 1, 1989, and Sept. 15, 1990, 305 patients at Los Angeles County/University of Southern California Women's Hospital were studied in a prospective, randomized fashion. Patients were initially identified as having oligohydramnios on admission on the basis of a four-quadrant amniotic fluid index of <5.0 cm at the time of admission.⁷ Patients either were

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in labor or were being admitted for induction of labor because of oligohydramnios. Membranes could be either intact or spontaneously ruptured on admission. Criteria for enrollment also included a singleton gestation, vertex presentation, and normal fetal heart rate baseline and variability. Exclusion criteria included moderate or severe variable decelerations, late decelerations, vaginal bleeding, fetal anomalies, and chorioamnionitis. Patients were not excluded because of the presence or absence of meconium. Once enrolled, patients were not excluded from the study, nor were they randomized to any other studies. Eligible patients who agreed to participate in the institutional review board—approved study were enrolled after providing written informed consent.

After enrollment, patients were prospectively randomized to a treatment group (amnioinfusion) or a nontreatment group. Randomization was done by computer randomization and sequentially numbered opaque, sealed envelopes. The ratio of treatment to nontreatment groups was 3:2. The 3:2 ratio was chosen to encourage enrollment. Study design included power calculations that were based on an α error of 0.05 to establish statistical significance and a β error of 0.20. Calculations showed that a minimum of 114 patients were required in each arm, on the basis of the differential rate of cesarean delivery because of fetal distress (13% vs 3%) and the difference in the incidence of amnionitis (20% vs 7%) as reported by Strong et al.5 in an earlier study completed at our institution.5

After adequate cervical dilation had been achieved, patients had an intrauterine pressure catheter inserted, through which those patients in the amnioinfusion group received an infusion of 500 ml normal saline solution at 37° C. Saline solution was infused by gravity

Table I. Patient characteristics

	Infusion gorup (N = 175)	Control group (N = 130)	p Value
Age (yr)	23.3 ± 3.9	23.0 ± 4.3	NS
Gravidity	1.7 ± 0.7	1.7 ± 0.8	NS
Parity	0.5 ± 0.6	0.4 ± 0.7	NS
Nulliparous women	104 (59.4%)	88 (67.7%)	NS
Multiparous women	71 (40.6%)	42 (32.3%)	NS
Gestational age (days)	290 ± 7.7	291 ± 8.1	NS
Initial cervical dila- tation (cm)	2.9 ± 1.0	3.1 ± 0.9	NS
Amniotic fluid index on admission	3.6 ± 0.7	3.4 ± 0.8	NS

NS, Not significant.

Table II. Method of delivery

	Infusion group	Control group	p Value
Normal spontaneous vaginal delivery	122/175 (69.7%)	66/130 (50.8%)	0.001
Forceps	17/175 (9.7%)	17/130 (13.0%)	NS
Vacuum	11/175 (6.3%)	11/130 (8.5%)	NS
Cesarean	25/175 (14.3%)	36/130 (27.7%)	0.006
Total operative deliveries	53/175 (30.3%)	64/130 (49.2%)	0.001

NS, Not significant.

flow over 20 to 30 minutes (15 to 25 ml/min). After the initial infusion, ultrasonography was performed to measure the amniotic fluid index and confirm adequate restoration of intrauterine fluid volume (amniotic fluid index, ≥10.0 cm). If the amniotic fluid index was found to be ≥10.0 cm, no further fluid was added. If the amniotic fluid index was ≥5.0 cm and <10.0 cm, an additional 250 ml was given, and if the amniotic fluid index was <5.0 cm, 500 ml was again given after intrauterine catheter placement was checked. Ultrasonography was repeated hourly, with the above-noted schedule followed to maintain an amniotic fluid index of ≥10.0 cm. Patients were allowed to labor, and on delivery cord gas values were obtained. Infants and parturients were monitored for signs of infection or other complications until discharge from the hospital.

It should be noted that the study was not blinded, because of the need for hourly ultrasonographic examinations and reinfusion in some patients. In an effort to eliminate bias, managing physicians were not the clinical investigators.

After completion of the study, data from all patients were included and reviewed. Tests for statistical comparison included the χ^2 , Mann-Whitney U, and Student t test.

Results

After enrollment and randomization, 305 patients were studied. One hundred seventy-five patients underwent amnioinfusion, and 130 patients served as a control group. Comparison of the treatment group versus the control group revealed no statistical differences between the groups with regard to age, parity, gestational age, initial cervical examination or admission amniotic fluid index (Table I).

In the infusion group patients received an average of 1.2 ± 0.5 infusions and an average volume of 554 ml (± 122 ml). Average increase in amniotic fluid index with the initial 500 ml infusion was 8.4 ± 1.4 cm. ·Twenty-three patients required more than one infusion, while 152 needed just the initial 500 ml bolus. In those requiring a second infusion, the average time to that infusion was 4.6 ± 1.6 hours. There were no infusion failures, although the intrauterine pressure catheter had to be replaced in three patients. No infusions had to be stopped because of fetal distress.

Comparison of the delivery methods between the infusion group and the control group shows significant differences in the overall incidence of cesarean section, total operative deliveries, operative deliveries for fetal distress, and cesarean section for fetal distress (Tables II and III).

Neonatal outcome revealed a significant difference in cord pH between the two groups, although 5-minute Apgar scores were not significantly affected (Table IV). Amnioinfusion did not appear to have any adverse effect on the fetus. None had seizures in the newborn period after the amnioinfusion, and there were no

Table III. Operative intervention because of fetal distress

	Infusion group	Control group	p Value
Forceps	4/175 (2.3%)	5/130 (3.8%)	NS ·
Vacuum	3/175 (1.7%)	4/130 (3.1%)	NS
Cesarean	7/175 (4.0%)	25/130 (19.2%)	0.0001
TOTAL	14/175 (8.0%)	34/130 (26.2%)	0.0001
No distress	161/175 (92.0%)	96/130 (73.8%)	0.0001

NS, Not significant.

Table IV. Neonatal outcome

	Infusion group	Control group	p Value
Birth weight (gm)	3545 ± 350	3459 ± 325	NS
Umbilical artery cord pH	7.27 ± 0.05	7.23 ± 0.09	0.0001*
No. with pH <7.20 Apgar scores <7	14/175 (8.0%)	39/130 (30.0%)	0.0001
l min	14/175 (8.0%)	45/130 (34.6%)	0.0001
5 min	6/175 (3.4%)	9/130 (6.9%)	ŃS

NS, Not significant.

*Student t test.

Table V. Infection

	Infusion group	Control group	p Value
Amnionitis	18/175 (10.3%)	9/130 (6.9%)	NS
Endometritis	7/175 (4.0%)	10/130 (7.7%)	ŇS
Length of hospital stay (maternal, days)	2.5 ± 1.1	3.0 ± 1.5	<0.01*
No. with stay >3 days	23/175 (13.5%)	37/130 (29.5%)	0.002
Length of hospital stay (infant, days)†	2.5 ± 1.1	3.9 ± 3.8	<0.001*
No. with stay >3 days	24/175 (13.7%)	45/130 (33.2%)	0.0001

NS, Not significant.

*Mann-Whitney U.

†Reflects maternal stay.

other newborn problems associated with the procedure.

The incidence of both amnionitis and endometritis was not significantly different between the infusion and the control group. Interestingly, the length of hospital stay was significantly longer in the control group, a reflection of the increased rate of operative deliveries observed in this group (Table V).

Comment

The role of amniotic fluid in providing a cushion for the fetal umbilical cord has now been suggested by several authors.^{5, 8} The results obtained in our study provide further evidence that the maintenance of adequate amniotic fluid volume is important during the intrapartum period. The apparent protection against cord compression seems to have a direct effect on both maternal and fetal outcome, and our results lend strong support to the technique of amnioinfusion as a prophylactic treatment for oligohydramnios in labor.

In a comparison of the methods of delivery (Tables II and III), it is readily apparent that the infusion group has a statistically significant increase in spontaneous vaginal deliveries and a subsequent decrease in operative deliveries. Operative intervention specifically because of fetal distress is also significantly reduced when one examines the overall rate (including forceps and vacuum) and when cesarean section alone is examined. Prior studies of cesarean section rates have suggested this trend but have not shown a statistical difference because of their smaller sample sizes.^{2, 5} This finding clearly translates to decreased maternal morbidity and a reduction in the number of cesarean deliveries. Of interest is the observation that operative vaginal deliveries because of fetal distress are higher in the study

population (both infused and noninfused patients) than in the general population at Women's Hospital. This is believed to reflect the complication of oligohydramnios in the study population. The observation that the rate did not return to normal in the infusion group suggests that in some patients oligohydramnios is a symptom of a larger problem, not simply corrected by amnioinfusion and restoration of a normal amniotic fluid index.

With regard to fetal outcome, our data show a significant improvement in umbilical artery cord pH in the amnioinfused group. One-minute Apgar scores also were improved, but the 5-minute scores were not statistically different. Our results are similar to those reported in prior studies2,5 and presumably reflect decreased stress in association with the elimination of repetitive variable decelerations.

Of concern in our institution was the possible implication of amnioinfusion with increased rates of amnionitis and endometritis. Strong et al.⁵ reported a 13% increase in infection in his preliminary study of infused patients. This enlarged study failed to show a statistical difference between the infection rates between the control and treatment group, and the differences noted were only 3%. Recent work published by Owen et al.6 has suggested that amnioinfusion may actually provide a protective effect against maternal infection. Our work was unable to confirm this finding.

Of great interest was the shortened length of hospital stay in the amnioinfusion group. While this finding is tied to the decreased rate of operative intervention for fetal distress, its importance is nonetheless diminished. In this era of rising medical costs, decreased hospital time for both mother and infant would seem to provide further incentive for using this technique.

In summary, this study shows that prophylactic amnioinfusion is a safe and simple technique that may be used for the treatment of oligohydramnios in patients in labor. Its benefits include a decrease in the rate of operative intervention for fetal distress, reduced rates of cesarean section, and decreased length of hospital stay. Amnioinfusion has no apparent adverse effects on the fetus or mother and does not appear to increase the rates of maternal infection.

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The second stage of labor: Factors influencing duration

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Prolonged second stage of labor is associated with increased perinatal mortality. Factors influencing second-stage duration are poorly understood. This study was undertaken to characterize those factors. A population of 473 nulliparous women and 491 multiparous women with spontaneous vaginal deliveries were analyzed extensively with history, physical examination (including clinical pelvimetry), labor and delivery data, and neonatal measurements. On the basis of stepwise multiple linear regression, epidural analgesia (p < 0.0001), active-phase duration (p < 0.0001), parity (p < 0.0001), height (p < 0.0004), birth weight (p < 0.0003), and station at complete dilatation (p < 0.027) predicted second-stage duration. The sum of their effect, however, accounted for <25% of the variability in second-stage length (total $R^2 = 0.233$), leaving 75% of the variance unexplained. (AM J OBSTET GYNECOL 1991;165:976-9.)

Key words: Labor, second-stage duration, spontaneous vaginal delivery

Prolonged second stage of labor is associated with increased perinatal morbidity and mortality. The definition of abnormal second-stage length (for example, >2 hours) is arbitrary. Classification of a second stage as normal or prolonged would be much more accurate if individual characteristics could be used to predict its length. Previous studies have associated parity and epidural analgesia with labor duration²⁻⁴; however, strength of correlation was not tested, and no other factors were considered. Some of these studies also included patients whose second stage was artificially shortened by operative delivery. St. 4

To create a model for prediction of second-stage length, a well-characterized population of patients with spontaneous vaginal delivery would be required. This study was undertaken to obtain such a population; to examine the influence of multiple maternal, fetal, and labor characteristics on second-stage duration; and to determine the relative contribution of each factor to the variability in second-stage length. The ultimate goal was to create a mathematic model for prediction of second-stage duration, on the basis of individual characteristics known at the onset of complete cervical dilation.

Material and methods

After protocol approval by the human subjects review boards of both hospitals, patients delivered between October 1987 and August 1990 at Humana

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Women's Hospital and Medical Center Hospital, San Antonio, were enrolled. Patient selection was based on consecutive deliveries, limited by the work schedule of the research personnel. Only women with viable singleton pregnancies were enrolled. Multifetal gestation, intratuerine fetal death, or operative delivery excluded the patient from further analysis. To be enrolled, the women had to have the ability to read and give written consent in English. A population of women with spontaneous vaginal deliveries was created.

During the postpartum hospitalization, three experienced research personnel conducted a chart review, interview, and physical examination of the mother (including clinical pelvimetry) and the neonate. The examiners were uniformly instructed on examination technique by one of the authors (E.R.N.). Interobserver variability was evaluated for clinical pelvimetry, with all observations correlating within 0.5 cm. Extensive data were collected on maternal, neonatal, and labor characteristics (Table I).

Labor management was at the discretion of the attending physician. In general, patients were examined every 1 to 2 hours unless clinically indicated (urge to push, change in fetal heart tracings, etc.). Second-stage duration was defined as the time from diagnosis of complete cervical dilatation to delivery. The active phase of labor was defined as the period from a cervical dilatation of 4 cm to complete dilatation. If the patient was admitted with the cervix dilated >4 cm, active-phase length was measured from admission to complete dilatation. Station was measured as centimeters above or below the ischial spines.

Prepregnancy weight and shoe size were based on patient history. Pregnancy weight gain equaled the difference between weight on admission and prepregnancy weight. Maternal pelvic measurements, made by means of calipers with the patient in lithotomy position, were the transverse outlet diameter (the inside width

Table I. Maternal, neonatal, and labor characteristics

Maternal history Prenatal and admission data Labor Age Weight gain Latent phase length Race Glucose screen Active phase length Parity Complications Station at complete dilatation Medical problems Hematocrit Second-stage length Prepregnancy weight Induction (?) Oxytocin Shoe size Gestational age Magnesium sulfate Prior deliveries Infant examination Epidural analgesia Other history Birth weight Intravenous sedation Paternal height Head circumference Antibiotics Prior infant's Length Delivery Birth weight Chest circumference Type of delivery Length Shoulder diameter Episiotomy Head circumference Apgar scores Perineal lacerations Physical examination Fetal head position Śubpubic angle Shoulder dystocia Biischial diameter Complications Measured foot size Care provider Maternal height Weight at delivery Cervical examination at admission

of the anterior ischial rami at the point of their inflection posteriorly and superiorly) and the diagonal from the right ischial ramus to the apex of the subpubic angle. The subpubic angle was calculated with trigonometric formulas.

All neonates were measured by standard techniques. Neonatal chest circumference was measured at the level of the nipples. Shoulder diameter was measured between the most lateral aspects of the acromion. Prior infants' measurements were obtained from the medical records.

· Univariate analysis was performed with the Pearson correlation and the Student t test for continuous data and χ^2 analysis for categoric data, with p < 0.05 considered significant. All significant factors were further examined with one-way analysis of variance and were entered as independent variables in multivariate analysis with stepwise multiple linear regression. Only firstdegree effects were considered.

Results

A total of 1071 women were approached for study inclusion. Of these, 51 refused to participate (4.8%); these women were more likely to be nulligravid (43/51) and to have painful perineal lacerations. The remaining 1020 women were entered into univariate analysis and were used to create the population characteristics. On entry to multiple regression, an additional 56 patients were excluded because of one or more missing data points, leaving a total of 964 women (473 nulliparous, 491 multiparous) for final analysis. Twenty percent of the nulliparous women (102) and 27% of the multiparous women (140) had cervical dilatation >4 cm on admission. The majority of those were 5 to 6 cm, with only 31 nulliparous women (6%) and 56 multiparous women (11%) admitted at >6 cm dilatation. Patients were enrolled from two hospitals (one public, one private) to create a diverse population. The only significant difference between the hospitals in the factors studied was a lower incidence of epidural analgesia at the public hospital (36% vs. 56%).

The population (subclassified as nulliparous, multiparous, and the total group) is characterized further in Table II. Univariate analysis identified parity (p < 0.0001), weight gain (p < 0.015), shoe size (p < 0.032), height (p < 0.001), transverse diameter of pelvic outlet (p < 0.001), subpubic angle (p < 0.037), epidural analgesia (p < 0.0001), activephase length (p < 0.0001), station at complete dilatation (p < 0.008), birth weight (p < 0.007), and head circumference (p < 0.006) as significantly correlated with second-stage duration. Table III illustrates the influence of selected factors on the length of second stage (the significance levels refer to comparison between groups for each factor). Multiple stepwise linear regression was performed with second-stage length as the dependent variable and the significant factors from univariate analysis as the independent variables. Six factors were identified as significant predictors. These factors and their relative contributions to the total variability in second-stage duration are displayed in Fig. 1. The influence of these factors varies between nulliparous and multiparous women and the total group. In all groups, however, the total R^2 was <0.25, with individual R² values ranging from 0.009 to 0.119. Thus the majority of the variability in second-stage length could not be explained by maternal, fetal, or labor characteristics.

Comment

The optimum maximal duration for the second stage of labor has always been controversial. In the nine-

Table II. Characteristics of study population

	Primiparous	Multiparous	Total
Maternal characteristics			
Age	21.7 ± 4.9	27.1 ± 5.6	24.4 ± 5.9
Parity	_ '	1.6 ± 1.0	0.8 ± 1.0
Height (cm)	161 ± 8	162 ± 7	161 ± 7
Weight at delivery (kg)	72.7 ± 14	75.5 ± 14	74.1 ± 14
Weight gain (kg)	11.9 ± 7.5	10.6 ± 7.1	11.3 ± 7.3
Maternal shoe size	7.2 ± 1.2	7.3 ± 1.1	7.3 ± 1.2
Infant characteristic			
Birth weight (gm)	3259 ± 477	3431 ± 491	3355 ± 492
Head circumference (cm)	34.0 ± 1.5	34.3 ± 1.5	$34.1 \pm 1.5^{'}$
Length (cm)	50.4 ± 2.4	50.5 ± 2.4	50.4 ± 2.4
Shoulder diameter (cm)	14.6 ± 1.0	14.8 ± 1.0	14.7 ± 1.0
Chest circumference (cm)	33.0 ± 1.9	33.6 ± 1.9 .	33.3 ± 1.9
Labor characteristic		•	
· Active phase (hr)	4.6 ± 3.6	2.9 ± 2.8	3.8 ± 3.3
Station, complete dilatation (cm)	$+0.9 \pm 1.1$	$+0.4 \pm 1.2$	$+0.6 \pm 1.1$
Epidural analgesia	53%	46%	50%
Oxytocin	42%	31%	37%
Second stage (min)	52.6 ± 41.8	22.8 ± 25.2	37.7 ± 37.5

Values are mean \pm SD.

Table III. Selected factors that influence second-stage duration

	Second stage (min)	p Value
Parity		
0 ′	52.6	< 0.0001
1	24.6	
2	22.7	
3 .	13.5	
Epidural	•	
Yes	48.5	< 0.0001
No	27.0	
Active phase length		
<1.5 hr	26.0	< 0.0001
1.5-2.9 hr	33.8	
3.0-5.4 hr	41.7	
>5.4 hr	49.3	
Weight gain		•
<10 kg	34.3	< 0.019
10-20 kg	38.9	
>20 kg	45.6	
Birth weight		
. <2500 gm	22.3	< 0.025
2500-2999 gm	35.2	
3000-3999 gm	38.9	
≥4000 gm	41.2	

teenth century the controversy stemmed from the preexisting opinion that the second stage should be allowed to continue indefinitely (because of fear of operative delivery) and the experience that maternal and fetal morbidity was proportional to second-stage length. In the midst of this debate, Hamilton, in 1861, created the limit of 2 hours for second-stage duration.

In 1952, Hellman and Prystowsky¹ found that infant mortality rose significantly when second-stage length passed 150 minutes. Their study, as well as Friedman and Knoll's⁶ data published in 1969, found a median

second-stage duration of 50 minutes in nulliparous women and 20 minutes in multiparous women.

The dogma of a 2-hour limit was attacked in 1977 by Cohen, on the basis of his study of 4000 nulliparous women. He found no increase in maternal or neonatal mortality with increased second-stage length as long as the fetal heart tracing remained normal and concluded that it was unwarranted to intervene simply because a time limit had been surpassed. In contrast, Roemer et al. suggested that the second stage should not be allowed to go beyond 45 minutes, on the basis of umbilical cord gas analysis. Katz et al., in 1987, found clinically significant decreases in umbilical arterial pH values as second-stage length increased. They called for a new evaluation of optimal second-stage duration.

In the 1980s, several studies demonstrated that epidural analgesia increased second-stage length.2-4 Kilpatrick and Laros² suggested that the second-stage limit for patients with epidural analgesia be extended to 3 hours. The concept that epidural analgesia might alter the anticipated second-stage length prompted our search for other factors that might improve our ability to predict second-stage duration. Our study attempted to create a model for prediction of second-stage duration on the basis of multiple maternal, neonatal, and labor factors. Our inability to identify the source of the majority of variability in second-stage duration thwarts attempts at mathematic modeling. The source of the remainder of variability must first be identified. Further investigation should be directed toward the powers of labor responsible for fetal descent, including uterine force, contractile pattern, and maternal pushing effort, and to the effects of minor aberrations of fetal position and pelvic architecture. Also, the effect of epidural anesthesia should be further evaluated as to the relative

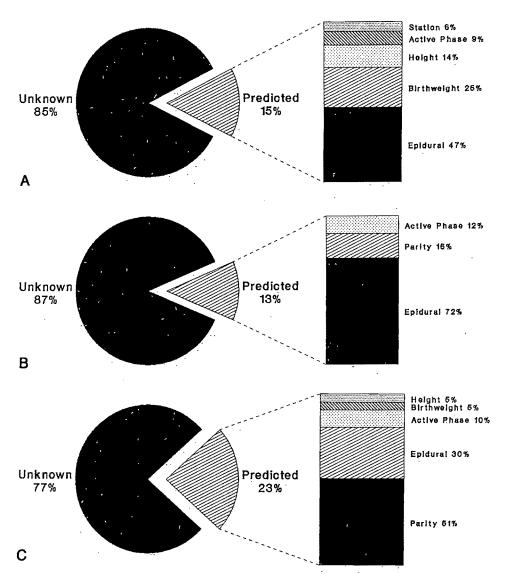


Fig. 1. Sources of variation in second-stage duration and their relative contributions to total variability on basis of multiple linear stepwise regression. A, Nulliparous women; total $R^2 = 0.151$ (n = 473); **B**, multiparous women; total $R^2 = 0.128$ (n = 491); **C**, total group; total $R^2 = 0.233$ (n = 964).

contributions of anesthetic type and density of blockade.

When a predictive model is developed, the anticipated second-stage duration for an individual can be calculated and a pathologic second-stage length can be established, on the basis of maternal and neonatal morbidity and mortality. Thus the timing of intervention for a prolonged second stage can be tailored to decrease operative intervention and improve maternal and neonatal outcome.

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A prospective study of two dosing regimens of oxytocin for the induction of labor in patients with unfavorable cervices

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The ideal regimen for induction of labor with oxytocin with respect to the magnitude and frequency of dosage changes has not been defined. In spite of few data regarding labor induction with an unfavorable cervix, the initial dose recommended by the American College of Obstetricians and Gynecologists is lower than that of other commonly used protocols. Eighty patients with unfavorable cervices and unruptured membranes, without evidence of labor, were randomized to one of two protocols and met criteria for data analysis. Patients in both protocols were given an initial dose of oxytocin of 2 mU/min. Patients in protocol A (n=32) then received incremental increases of oxytocin of 1 mU/min at 30-minute intervals, while those in protocol B (n=48) received incremental increases of 2 mU/min at 15-minute intervals. Induction failures were higher among patients on protocol A (31% vs 8%, p<0.05). Patients on protocol B had shorter times to delivery (mean = 10 hours 57 minutes vs 8 hours 3 minutes; p<0.05). The number of operative deliveries were similar regardless of protocol. There were no significant differences (p=NS) among groups and protocols in maternal and fetal complications, cesarean section rate, and uterine hyperstimulation. In this population a more aggressive protocol may lead to fewer induction failures and shorter induction-to-delivery intervals. (Am J Obstet Gynecol. 1991;165:980-4.)

Key words: Oxytocin, induction of labor, unfavorable cervix

The induction of labor is defined as the initiation of uterine contractions before the spontaneous onset of labor by medical or surgical means, or both, for the purpose of accomplishing delivery.1 Dilute intravenous oxytocin infusion remains the only agent approved by the Food and Drug Administration for induction at the present time. Numerous induction protocols exist involving different initiation doses, rates of increase, and time intervals between increases. An infusion beginning with 0.5 to 1.0 mU/min with incremental increases of 1 mU/min at 30- to 60-minute intervals is currently recommended by the American College of Obstetricians and Gynecologists. The recommendation derives its basis principally from data reported by Seitchik and Castillo² obtained from patients who had presented in labor and received oxytocin for augmentation of labor. Hauth et al.3 subsequently demonstrated that significantly less oxytocin and less uterine activity are required for successful augmentation of labor versus induction of labor. Accordingly, information regarding the amount of oxytocin required and uterine contraction forces obtained during labor augmentation may have little or no relevance as regards labor induction.

Delivery is often indicated in women with unfavorable cervices, especially in prolonged pregnancies, which constitute 7% to 12% of all gestations. A Ecommendations for induction management have been based on retrospective, uncontrolled populations or patients undergoing augmentation rather than induction of labor. The purpose of our study was to compare the efficacy and safety of two commonly used induction regimens in a prospective, controlled manner in patients not in labor and with unfavorable cervices.

Material and methods

The study population consisted of women admitted to the labor and delivery suite for the induction of labor. The majority (94%) of women undergoing induction had postterm pregnancies (≥42 weeks' estimated gestational age). Women were examined before enrollment and were included only if the results of cervical examination were unfavorable (dilatation ≤2 cm and a Bishop score ≤6) and there was no evidence of regular uterine activity or evidence of rupture of fetal membranes. Approval for this study was granted by the institutional review board. After informed consent was obtained from each patient, an

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unmarked, sealed envelope was opened, which assigned the patient to one of the protocols (Table I). Women presenting for induction of labor were excluded from the study if they had a malpresentation, placenta previa, active herpes infection, or hypertensive disease necessitating magnesium sulfate treatment or if a deviation from dosing protocol had occurred.

Regardless of protocol, patients receiving oxytocin were monitored in a similar fashion.6 A baseline fetal heart rate tracing was obtained before initiation of oxytocin. Standard recording of vital signs included continuous fetal heart rate monitoring and uterine contractions and serial 15-minute recording of maternal blood pressure, pulse, and fetal heart tones. Contraction intensity, duration, and frequency and maternal fluid intake and output also were recorded. Oxytocin dosages were increased until an adequate labor pattern was achieved. An adequate labor pattern was defined as labor resulting in cervical change. Amniotomy was performed only after cervical change occurred and the patient was diagnosed as having progression beyond latent-phase labor. After rupture of membranes, a fetal scalp electrode and intrauterine pressure catheter were placed. In the absence of cervical change oxytocin was increased per each particular protocol until >200 montevideo units per 10 minutes was achieved.7 At 8 to 10 hours after the start of induction, if cervical dilatation or spontaneous rupture of membranes had not occurred and there was no evidence of fetal distress or maternal illness, the induction was considered failed.

Age, race, parity, estimated gestational age, height, weight, indication for induction, Bishop score, cervical examination result, and medical history were recorded for all patients. Other potential confounding variables such as birth weight and method of anesthesia also were compared. Duration of the first and second stages of labor were recorded. The maximum doses of oxytocin were noted.

Maternal outcome parameters tracked included the frequency of operative deliveries, chorioamnionitis, postpartum endometritis, and postpartum hemorrhage. Neonatal outcome as reflected by Apgar scores, umbilical cord gas values, pH, infection rate, and hospital stay also were compared.

Statistical analysis was performed with the Mann-Whitney U test, χ^2 contingency tables, and Fisher's exact test as indicated.

Results

The study included 80 women, 32 on protocol A and 48 on protocol B. An additional eight patients were excluded from protocol A and two from protocol B because hypertensive disease necessitating magnesium sulfate prophylaxis. Six patients were excluded from protocol A because of a deviation in dosing protocol.

Table I. Induction protocols in 80 women with unfavorable cervices

	Protocol A $(n = 32)$:	$\begin{array}{c} Protocol\ B\\ (n=48) \end{array}$
Initial dose of oxytocin (mU/min)	2	2
Increment increases (mU/min)	1	2
Time interval of increases (min)	30	15
Maximum dose (mU/min)	40	40

Table II. Maternal demographics

	$Protocol\ A (n = 32)$	Protocol B (n = 48)
Maternal age (yr, mean ± SD)	24.3 ± 3.6	24.7 ± 3.2
Height (in, mean ± SD)	63.8 ± 3.6	64.2 ± 2.7
Weight (lb, mean ± SD)	175 ± 26	172 ± 27
Nulliparous (No.)	15	22
Weeks' gestation (No.)		
≥42	31	44
40-42	1	4

p = NS.

Protocol A included 31 postdate pregnancies and one case of gestational diabetes; 15 of these women were nulliparous and 17 were parous. Protocol B included 44 women with postdate pregnancies, one with chronic hypertension, two with gestational diabetes, and one with suspected macrosomia. Protocol B consisted of 22 nulliparous and 26 parous women.

Comparisons of maternal age, parity, estimated gestational age, height, and weight were similar between protocols (Table II). There were no significant differences in the use of epidural anesthetics in labor between the two protocols (25% in protocol A and 27% in protocol B).

There were 10 (31%) induction failures with protocol A compared with 4 (8%) with protocol B (p < 0.05). Times from onset of induction to completion of the first and second stages of labor are summarized in Table III. Patients on protocol B had a significantly (p < 0.05) shorter time to delivery. The mean induction-to-delivery time in nulliparous women on protocol A was 15 hours 18 minutes versus 9 hours 16 minutes for those on protocol B (p < 0.05). There was also a significant difference in parous women (10 hours 54 minutes in group A vs 8 hours 2 minutes in group B; p < 0.05).

The number of times the oxytocin had to be stopped or decreased because of hyperstimulation and fetal heart rate abnormalities is depicted in Table IV. The maximum dose of oxytocin used to achieve adequate labor was significantly higher in protocol B than pro-

Table III. Duration of induction

	Protocol A $(min, mean \pm SD)$	$\begin{array}{c} Protocol\ B\\ (min,\ mean\ \pm\ SD) \end{array}$	Difference
Nulliparous	 	· · · · · · · ·	
Time to 2nd stage	723 ± 420	626 ± 276	
Duration of 2nd stage	105 ± 77	89 ± 33	
Total time to delivery	918 ± 420	556 ± 318	<i>p</i> < 0.05*
Parous			•
Time to 2nd stage	600 ± 342	448 ± 170	
Duration of 2nd stage	54 ± 68	35 ± 39	
Total time to delivery	654 ± 370	482 ± 190	p < 0.05*

^{*}Mann-Whitney statistical analysis.

Table IV. Incidence of hyperstimulation

	No. of episodes			•
	0	1	2	≥3
Protocol A $(n = 3)$	 32)			
Nulliparous	11	2	1	1
Parous	10	6	0	1
Protocol $(n = 48)$				
Nulliparous	10	5	2	5
rumparous				

p = NS.

tocol A (25.5 \pm 9 vs 14.4 \pm 7 mU/min; p < 0.05). There were no emergent deliveries in either group or any infants with evidence of umbilical arterial or metabolic acidemia.

Maternal complications, specifically the number of operative midpelvic and abdominal deliveries, chorioamnionitis, postpartum endometritis, and postpartum hemorrhage, were not significantly different between protocols (Table V). Infant demographics were also similar for birth weight, low Apgar scores, ominous cord gases, and neonatal infections (Table VI).

Comment

The optimal regimen of oxytocin for induction of labor, particularly in patients with unfavorable cervices, is unknown. One of the most frequent indications for induction of labor is a gestation of ≥42 weeks. The likelihood of achieving vaginal delivery can be predicted by the Bishop score. With a score of ≥ 9 , >50%were delivered within 5 days without intervention.8 Hayashi considered a pelvic score >6 to equate with successful induction and vaginal delivery.1 The rate of successful inductions in patients with scores <6 goes down significantly. The incidence of cesarean delivery in patients who are postdates and have unfavorable cervices varies from 6% to 51%.9-12 In large part this is accounted for by whether the patient with an unfavorable cervix is allowed sequential inductions or is committed to delivery the same day by amniotomy. Accordingly, we have previously reported a system of trial

inductions of labor in postdate gestations with intravenous oxytocin beginning at 2 mU/min and increasing by 2 mU/min every 15 minutes until an adequate labor pattern is achieved.12

Recently, it was recommended that lower doses of oxytocin and longer intervals between dose increases be used for induction of labor. 1, 2, 13, 14 The initial study of Seitchik et al.15 involved only women at ≥38 weeks' gestation who had already entered labor and required oxytocin for augmentation of labor. They reported a higher incidence of delay in time until the second stage in patients receiving higher doses of oxytocin with shorter intervals between dosing increases in association more transient curtailments of infusion out of concern for hyperstimulation and fetal distress. In this retrospective review they concluded that concerns for discontinuing the medication were rarely of pathophysiologic significance. Seitchik et al.15 subsequently demonstrated that approximately 40 minutes is required for any particular dose of oxytocin to reach steady state. Hauth et al., susing an induction regimen with 1 to 2 mU/min increases at intervals of \geq 15 minutes, reported that 29% of women requiring oxytocin induction received >20 mU/min, whereas only 14% of women undergoing augmentation required >10 mU/min,3 thus suggesting that the optimal regimen of oxytocin for augmentation versus induction may be quite different.

Some recommend increasing oxytocin at 15-minute intervals on the basis of its short plasma half-life.16, 17 Foster et al.¹⁸ retrospectively compared 15-minute versus 30-minute dosing intervals in patients with mean cervical dilatation >4 cm undergoing augmentation. These authors reported no significant differences in time to delivery, cesarean section rates, or complications between regimens. Blakemore et al. 14 contrasted hourly versus 15-minute dosing intervals. Their study population included patients with various initial examinations. Although the average induction-to-delivery times were 1 hour 40 minutes shorter in the 15-minute group, the authors assumed a normal distribution of data and concluded this was not a significant difference.

Table V. Maternal complications

1 .	Midforceps	Cesarean section	Chorioamnionitis	Postpartum hemorrhage
Protocol A	4			
Nulliparous	- 1	1	1	0
Parous	1 .	` 0	1	. 0
Protocol B		•		
Nulliparous	. 2	3	2 · ′	0
Parous	0 .	2	0	0

p = NS.

Our data suggest advantages and disadvantages for each protocol. Protocol A required a lower maximum dose of oxytocin to achieve vaginal delivery in those patients with progression beyond the latent phase. However, we saw no adverse consequences such as antidiuresis, postpartum hemorrhage, or uterine rupture in patients receiving on average of 25 versus 14 mU/min of oxytocin. Patients in protocol A also required less intervention because of hyperstimulation. However, none of these episodes regardless of protocol necessitated emergent delivery, and none of these infants were born with a metabolic acidosis. Treatment consisted of a combination of supplemental oxygen, lateral tilt of the patient, or a transient discontinuation of infusion.

More patients had progression beyond the latent phase in an 8- to 10-hour period on protocol B as evidenced by fewer failed inductions. Time to delivery also was shorter for patients on protocol B (with the Mann-Whitney U test and not assuming a normal distribution of data). In the clinical setting of postdate pregnancy with an unfavorable cervix, a willingness to offer sequential inductions and a more aggressive protocol may optimize time to delivery without increasing the number of operative deliveries. The shorter time in labor and lower incidence of failed inductions will lead to an overall decrease in hospital stay and cost. Differences in our results compared with those of previous investigations may merely reflect differences in study populations. Our results imply that the application of data and recommendations derived from augmentation of labor in patients with favorable cervices to women with unfavorable cervices undergoing induction may not be valid. Although the higher incidence of hypertensive disease on protocol A was not significant, this combined with protocol dosing errors may have provided a source of exclusion bias. Nevertheless, because major complication rates are similar, the various regimens remain acceptable and should be available at the discretion of the individual clinician.

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Table VI. Neonatal demographics and complications

	$Protocol\ A (n = 32)$	$\begin{array}{c} Protocol\ B\\ (n=48) \end{array}$
Birth weight (gm, mean ± SD) Apgar score (No.)	3623 ± 459	3670 ± 516
≤3 at 1 min	. 0	1
≤6 at 5 min	1	1.
Metabolic acidosis* (No.)	0	0
Infection (No.)	1	0

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Do catechol estrogens participate in the initiation of labor?

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To assess the role of catechol estrogens in the initiation of labor, we compared the levels in amniotic fluid during the second and third trimesters and from women undergoing cesarean section at term not in labor and those with spontaneous labor at term. Catechol estrogen concentrations in amniotic fluid increased significantly with the progress of pregnancy. Further, concentrations (mean \pm SE) were significantly higher in spontaneous labor at term (468.6 \pm 29.5 pg/ml) compared with those obtained during cesarean section (242.6 \pm 22.3 pg/ml) at term not in labor. We suggest that catechol estrogens, through their stimulating effects on prostaglandin synthesis, participate in the initiation of labor. (AM J OBSTET GYNECOL 1991;165:984-7.)

Key words: Catechol estrogens, amniotic fluid, labor, gestational age, prostaglandin

The mechanism of the onset of labor in humans still remains incompletely understood. As opposed to ruminants, human parturition is not preceded by dramatic changes in the concentration of progesterone or estrogens in maternal circulation or changes in cortisol levels in the fetal circulation. In spite of the differences in the biochemical events occurring before labor among species, the central role of prostaglandins in the normal onset and progression of labor in all mammalian species studied so far has become evident in recent years.

Whereas the factors initiating and regulating prostaglandin synthesis in the fetal membrane and the uterine wall remain to be elucidated, catechol estrogens, major metabolites of estrone and estradiol in both laboratory animals and humans, 3,4 can be a significant factor in this regard. Recent studies have shown that catechol estrogens increase the production of prostaglandins even more than does the parent compound in rat

and human uterus,5 intrauterine tissues at parturition,6 and rabbit blastocysts and endometrium.7 As potent competitive inhibitors of catechol-O-methyltransferase, catechol estrogens potentiate the lipolytic effect of epinephrine in releasing arachidonic acid from phospholipids.8 It is interesting to note that catechol-O-methyltransferase, which is the enzyme of prime importance in the metabolism of catechol estrogens and the catecholamines, has been found to be very high in human decidua vera tissue during pregnancy, reaching maximum levels at term.9 The conversion of estrogens to catechol estrogens in human uterus10 and placenta11 and in a host of other tissues including the fetal brain and pituitary⁸ make catechol estrogens an important factor to be considered in the mechanism of parturition. We have previously shown that umbilical venous and arterial levels of catechol estrogens are significantly higher at vaginal delivery than at abdominal delivery, without any significant difference in the maternal peripheral plasma content between the two modes of delivery.12 We have also reported recently that urinary catechol estrogens excretion in rats increases during pregnancy, reaching maximum levels before labor.18

In view of these considerations, our investigation was undertaken to evaluate further the status of catechol estrogens in amniotic fluid during pregnancy and at different modes of delivery. Results show that levels of catechol estrogens increase with the progress of preg-

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nancy and are significantly higher at vaginal delivery in comparison with those obtained at abdominal delivery not in labor.

Material and methods

Fifty-four healthy women who gave informed consent (21 to 36 years old) were included in our study, which was approved by the institutional review board. Amniocentesis was performed as a part of clinical care. Amniotic fluid was collected during the second trimester $(17.1 \pm 1.2 \text{ weeks})$ of pregnancy for fetal karyotyping (n = 7) and from third-trimester patients $(36.1 \pm 0.9 \text{ weeks})$ for determination of fetal lung maturity (n = 9). Samples of amniotic fluid (38.8 ± 1.5) weeks) were obtained during cesarean section (not in labor) by needle aspiration through the intact membrane after the uterine wall was opened (n = 18) and from women in active labor (39.9 \pm 1.3 weeks) from a pressure catheter introduced at the time of amniotomy (n = 20). Patients in the study group were free of medical and obstetric complications. Oxytocin, narcotics, anesthetic agents, and antibiotics were not used before delivery. Continuous epidural analgesia was provided for both modes of delivery. The fluid was collected in sterile containers containing 0.5 ml of 5% aqueous ascorbic acid to ensure the stability of catechol estrogens and stored at -20° C until analyzed.

Radioactive and inert 2-hydroxyestrone was synthesized, purified, and stored as described by us.4 Organic solvents were purchased (glass distilled) from Burdick and Jackson Labs, Inc. (Muskegon, Mich.). The phosphate-saline solution buffer (0.1 mol/L, pH 7.2; 0.9% sodium chloride, 0.1% sodium azide, and 0.1% globulin fraction II) used in the radioimmunoassay contained 0.05% ascorbic acid for the protection of the catechol estrogens.4

Dextran-coated charcoal was prepared by adding 0.5 gm dextran T-70 (Pharmacia Fine Chemicals, Piscataway, N.J.) to 5 gm Norit A (Fisher Scientific Company, Pittsburgh) in 40 ml buffer and was diluted to 10 mg charcoal/0.2 ml before use. Radioactivity was counted in 10 ml Liquiscint (National Diagnostics, Somerville, N.J.). Radioimmunoassay was performed as previously described by us4, 12 with minor changes. Samples of 1 ml aliquots were extracted with 10 ml methylene chloride that was washed with 2 ml bicarbonate buffer and 10% acetic acid. The organic extract was evaporated to dryness under a stream of nitrogen. Catechol estrogen was then quantitated with a 5% aliquot of the extract, in duplicate, by radioimmunoassay. Water blanks and control samples were also included in each assay. Blank values were subtracted before final computation. Recovery of added radioactive 2-hydroxyestrone was $72.3\% \pm 7.3\%$ (mean \pm SD). Water blanks were 3.4 ± 1.9 pg (mean \pm SD). Sensitivity, defined as the mean of the blank value plus 2 SD, is 7.2 pg per tube.

Table I. Parallelism tests for catechol estrogen analysis in amniotic fluid (picograms per milliliter)*

Dilution	Observed value	Expected value (% difference)
0	375	375
1:2	200	188 (+6)
1:3	108	125 (-13.6)

^{*}Data yielded a correlation coefficient of r = 0.99.

The specificity of measurements of catechol estrogens has been reported in our previous publications.4.12 For this study samples were further subjected to parallelism tests by assaying undiluted and diluted samples (Table I). The correlation coefficient (0.99) of parallelism tests indicates that the relative rankings of the two data sets involved are nearly perfect. The intraassay and interassay coefficients of variation were 5.8% and 6.7%, respectively.

In our study no chromatographic separation was performed before radioimmunoassay; thus the results include 2-hydroxyestrone and 2-hydroxyestradiol because both cross react 100% with the antisera.4 Statistical comparison of the mean level of catechol estrogens across the four study groups was made through analysis of variance. Subsequent pairwise comparisons of groups were made through the Tukey multiple comparison procedure.

Results

Levels of catechol estrogens in amniotic fluid during pregnancy and in amniotic fluid obtained during both modes of delivery are depicted in Fig. 1. The gestational age between two modes of delivery was not statistically different. Levels of catechol estrogens differ significantly (p < 0.001) across the four study groups. Multiple comparisons show that levels of catechol estrogens (mean \pm SE) increase significantly (p < 0.001) in the third trimester (208.5 \pm 22.4 pg/ml) compared with the levels observed in the second trimester (65.0 \pm 9.5 pg/ml). A sharp rise in the level of catechol estrogens was observed (p < 0.001 pg/ml) during spontaneous labor at term (468.6 \pm 29.5 pg/ml) compared with that of the third trimester and at cesarean section $(242.6 \pm 22.3 \text{ pg/ml})$. There was no significant difference in levels observed between the third trimester and cesarean section at term.

Comment

Concentrations of catechol estrogens in amniotic fluid increase significantly with the progress of pregnancy, reaching maximum at term. There is no significant difference between the levels in the third trimester and those obtained at cesarean section at term. How-

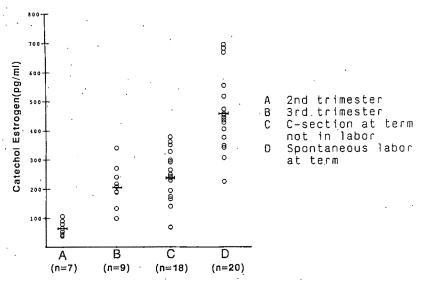


Fig. 1. Catechol estrogen concentrations (picograms per milliliter) during pregnancy and at different modes of delivery. Mean indicated by *horizontal bar. A* versus B, p < 0.001; B versus D, p < 0.001; B versus D, p < 0.001.

ever, a dramatic increase in concentration of catechol estrogens is observed in amniotic fluid collected at vaginal delivery (Fig. 1). Our data corroborate our previous findings of a significantly elevated concentration of catechol estrogens in cord blood at vaginal delivery.12 Significantly higher levels in both cord blood and amniotic fluid at vaginal delivery in comparison with those obtained at abdominal delivery would suggest that before or at the onset of labor there is an increase in production of catechol estrogens. Furthermore, since there is no significant difference in maternal peripheral plasma levels of catechol estrogens between the two modes of delivery,12 the increased production is perhaps localized to the fetoplacental complex, including fetal membranes. We have previously suggested that because of the high metabolic clearance rate, catechol estrogens in the peripheral circulation are not able to play a significant role in the reproductive processes.14 If there is any intrinsic role for catechol estrogens, then it is through their formation in the tissues in which they act.14, 15 Indeed, the absence of perceptible changes in progesterone and estrogen concentrations in the maternal peripheral plasma, either at term or in association with preterm labor,16,17 prompted investigators to study the formation of steroids within the intrauterine tissues and consequent paracrine regulation of prostaglandin production. 18, 19 Fetal membranes and decidua are capable of producing estrone and estradiol, and the activity of the enzyme estrone sulfatase in decidua and chorion increases substantially with the onset of labor.20 Thus the supply of unconjugated estrone may result in increased formation of catechol estrogens in the fetal membranes. Alternatively, the increased accumulation of catechol estrogens in amniotic fluid may be due to fetal urinary excretion. It is possible that as the fetus matures there is increased excretion of catechol estrogens into the amniotic fluid, reaching a maximum before the onset of labor. Catechol estrogens in turn, by transmembrane diffusion, directly or indirectly augment the synthesis of prostaglandin in the fetomaternal lining, leading to the initiation of labor and delivery. The report on the existence in fetal urine of a stimulatory substance of small molecular weight for prostaglandin synthesis²¹ strengthens such a proposition.

Catechol estrogens may also indirectly stimulate prostaglandin formation. Catecholamines are present in amniotic fluid in increasing concentration during late pregnancy.22 They may stimulate adenylate cyclase and influence prostaglandin production through β-adrenergic receptors present in the amnion tissue.23 Because catechol estrogens are potent competitive inhibitors of catechol-O-methyltransferase, they can protect catecholamines from inactivation, thereby potentiating and prolonging the action of these substances. The existence of protein-stimulating and protein-inhibitory substances in amniotic fluid for prostaglandin synthesis has been reported.18 Wilson et al.24 have recently described the isolation and purification of a protein from amniotic fluid that inhibits release of arachidonic acid from human decidual cells. The functional relationship of catechol estrogens with this and other inhibitory substances in amniotic fluid is not known.

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The effect of fetal movement counting on maternal attachment to fetus

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The effect of fetal movement counting on maternal attachment to fetus was investigated in 213 women with uncomplicated singleton pregnancies at 28 to 32 weeks' gestation. Women were randomized into those who counted fetal movements using the Sadovsky (n=63) or Cardiff (n=62) charts and controls (n=88). After 1 month of fetal movement counting, the Cranley 24-item scale with five subscales was used as a measure of maternal-fetal attachment. Univariate analysis revealed a statistically significant increase in total attachment scores and in each of the five attachment subscales among women who counted fetal movements (p<0.001). Tukey's studentized range test confirmed significant differences between each of the Sadovsky and Cardiff groups compared with controls (p<0.05). Our study suggests that fetal movement counting may enhance the maternal-fetal attachment process. (AM J OBSTET GYNECOL 1991;165:988-91.)

Key words: Maternal-fetal attachment, fetal movement counting

Maternal attachment to the fetus has been defined as the mother's affiliation and interaction with her unborn fetus. It develops during pregnancy as a result of dynamic psychologic and physiologic events and may be an important prerequisite to successful mother-infant adaptation. ^{2, 3}

Factors that influence the maternal-fetal attachment process are still being explored. Ultrasonographic examination, by providing a visual representation of the fetus in the early weeks of pregnancy, was shown in one study to promote maternal feelings of attachment.4 Quickening has also been demonstrated to be positively related to both prenatal and postnatal attachment behaviors. 5.6 With the onset of fetal movement, the nesting process appears to begin.7 Thus fetal movement may be a developmental variable influencing maternalfetal attachment. Hence, it has been suggested that helping mothers become more attuned to the occurrence of fetal movement may facilitate feelings of attachment.⁵ The purpose of this study was to examine the effect of fetal movement counting on maternal-fetal attachment. The specific research questions were: (1) What is the effect of fetal movement counting during the third trimester of pregnancy on maternal-fetal attachment? (2) Is there a difference between two methods of fetal movement counting (Sadovsky and Cardiff) on maternal-fetal attachment scores?

Material and methods

Two hundred thirteen pregnant women attending a prenatal clinic at the Bronx Municipal Hospital Center, Bronx, New York, were recruited and gave informed consent. The protocol was approved by the institutional review committee. All subjects had normal, uncomplicated singleton pregnancies. The gestational age ranged between 28 and 32 weeks of pregnancy. In an effort to use the two most common fetal movement counting methods, both the Sadovsky chart⁸ (counting three times a day after meals) and the Cardiff chart9 (counting the first 10 movements each morning) were used. Women were allocated to groups by a computergenerated random list. Women were randomized into those who counted fetal movements using the Sadovsky (n = 63) or Cardiff (n = 62) charts and controls (n = 88). Compliance was determined by number of days counted. Women in the control group did not count their fetal movements. Instructions were read to each participant from a printed script to ensure uniformity of counseling. All subjects completed demographic questionnaires. After 1 month of fetal movement counting, the Cranley 24-item scale with five subscales was used as a measure of maternal-fetal attachment. All women in the three groups completed the Cranley scale.

The Cranley maternal-fetal attachment scale consists of 24 Likert-type items describing baby-related thoughts and actions of expectant mothers. The Likert-type items comprised five responses to each question.

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Table I. Demographic characteristics of women by randomized group

	Group A	(n=63)	Group B	Group B $(n = 62)$		(n = 88)
Variable	-No.	%	No.	%	No.	%
Age (yr)						
17-20	22	34.9	17	27.4	19	21.6
21-25	20	31.7	25	40.3	32	36.4
26-30	12	19.0	8	12.9	27	30.7
≥3 İ	9	14.3	12	19.4	10	11.4
Parity						
Nulliparous	35	55.5	31	50.0	33	37.5
Multiparous	28	44.5	31 31	50.0	55	62.5
Race						
Black	23	36.5	15	24.2	21	23.9
White	15	23.8	15	24.2	15	17.0
Hispanic	23	36.5	32	51.6	48	54.5
Other	2	3.2	0		4	4.5
Education						
≤8 yr	7	11.2	1	1.6	14	15.9
9-12 yr	41	65.1	45	72.6	66	75.0
>12 yr	15	23.8	16	25.8	8	9.1
Marital status						
Married	29	46.8	23	37.1	32	36.3
Unmarried	33	53.2	39	62.9	56	63.6

Subjects were asked to circle one answer only. The responses are arranged on a five-point response set as follows: most of the time, frequently, sometimes, rarely, never. The scale is scored by assigning values to the responses (most of the time is 5, never is 1) and summing the item values. One item, "I feel that my body is ugly," has reversed scoring. The subscales are designed to measure (1) differentiation of self from fetus, (2) interaction with the fetus, (3) attributing of characteristics to the fetus, (4) giving of self, and (5) maternal role-taking. Internal consistency reliability (Cronbach's a) for the entire scale has been reported by Cranley¹ to be 0.85 and by Mercer et al.¹⁰ to be 0.80. All of the subscales were reported1 to be positively associated with the total scale (r = 0.61 to 0.83).

Results

Most of the women were Hispanic (48.1%), unmarried (60.8%), and unemployed (77.1%). The mean age was 24, with a range of 17 to 37 years. The mean years of education was 11.09. All subjects came from the Bronx borough in New York City, and the majority were multiparous (53.3%). Seventy-eight percent of those who counted completed the fetal movement graph on a daily basis. There was no statistically significant difference observed in compliance among women using the Sadovsky versus Cardiff

Table I presents the demographic characteristics of the women by randomized group. At least 65% of the women in each group had between 9 and 12 years of education.

Univariate analysis compared the total and subscale

maternal-fetal attachment scores for women who counted fetal movement versus women who did not count (Table II). There was a statistically significant increase in total attachment scores and an increase in each of the five attachment subscales among women who counted their fetal movements (p < 0.0001). Both total and subscale maternal-fetal attachment scores were lower in the control group when compared with groups A and B.

Tukey's studentized range test confirmed significant differences between each of the Sadovsky and Cardiff groups compared with controls. There were significant differences (p < 0.05) in total and in each of the subscale attachment scores between group A versus group C and between group B versus group C. There were no significant differences in attachment scores between groups A and B.

Linear regression modeling was performed to test any interaction effects of maternal demographic variables on attachment scores. None of the variables presented in Table I was statistically significant. However, there was a trend correlating lower educational levels and nulliparity with decreased attachment scores. A larger sample size is needed to determine the true effects of these demographic variables on maternal-fetal attachment.

Comment

This study was conducted to examine the effect of fetal movement counting on maternal-fetal attachment. The authors did not think that different methods of fetal movement counting would have different effects on attachment scores, but rather the process of count-

Table II. Comparison of maternal-fetal attachment scores for women who counted (groups A and B and both groups combined) versus women who did not count (group C)

•	Grov	ι ρ Α*	Grov	ıp B†		d groups ! B‡, §	Ĝro	ир С
Score	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total score	3.79	0.409	3.81	0.372	3.80	0.390	2.97	0.834
Subscale scores								
Role-taking	4.40	0.436	4.39	0.501	4.39	0.468	3.23	1.141
Differentiation of self from fetus	4.24	0.672	4.31	0.651	4.27	0.643	3.21	1.122
Interaction with fetus	3.05	0.794	3.07	0.708	3.06	0.751	2.53	0.778
Attributing charac- teristics to fetus	3.52	0.614	3.51	0.547	3.51	0.580	2.67	0.894
Giving of self	4.00	0.659	4.06	0.539	4.03	0.599	3.36	0.887
TOTAL SAMPLE SIZE	(33	. (62	1:	25	8	8

^{*}All respective total and subscale scores between groups A and C were significantly different at p < 0.05 (F value = 3.88).

ing, regardless of the method used, may enhance maternal-fetal attachment. To demonstrate that it is the process rather than the method, we used two different fetal movement counting techniques. Our study demonstrates that women who counted their fetal movements had higher attachment scores compared with controls. This suggests that fetal movement counting enhances maternal-fetal attachment. It is possible that through movement the fetus may be engaging the mother in a set of behaviors that propel her feelings of affiliation and interaction with the fetus.

Originating in the psychologic work of Deutsch¹¹ and Bibring et al.12 the process of maternal-fetal attachment has been described as fundamental in preparing the mother for the real infant and fortifying her with the emotional energy she will need in the mothering role. Benedek¹³ supported a model of symbiosis between mother and fetus with each member contributing to the emergence of motherliness. In delineating the developmental stages of the pregnancy experience, Caplan¹⁴ theorized that the event of quickening assists the mother to begin conceptualizing the fetus as a separate being. Leifer15 identified several maternal behaviors indicative of maternal-fetal interaction, including talking to the fetus, offering the fetus food, and calling the fetus by a name. Brazelton¹⁶ suggested that the communicative relationship between mother and fetus in utero affects the rhythm of the mother-infant dyad after birth. He considered the baby to be an active participant in the attachment process and demonstrated the reciprocal nature of maternal-infant behavior. It now appears that this reciprocity begins before birth. With awareness of fetal movement, the psychologic process of attaching to the fetus seems to be hastened.

It is conceivable that enhancing the maternal-fetal attachment process may in turn improve patient compliance with prenatal health care, facilitate maternal adjustment during pregnancy, and have positive longterm effects on mother-child attachment. A child's secure attachment to the mother has been shown to be positively correlated with the child's exploration ability, problem-solving capability, sociability, and control in the preschool years.17 In that the attachment process is conceptualized as a complex life span phenomenon, additional studies are needed to determine whether promoting maternal-fetal attachment through techniques such as fetal movement counting has any longterm favorable consequences on mother-child relationship. The results of our study should be regarded as tentative. Caution is suggested before generalized recommendations involving regimental fetal movement counting for the purpose of enhancing the maternalfetal attachment process.

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[†]All respective total and subscale scores between groups B and C were significantly different at p < 0.05 (F value = 3.05).

[‡]All respective total and subscale scores between groups A + B and C were significantly different at p < 0.0001 (F value = 46.47). §There was no statistically significant difference between groups A and B.

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A randomized, double-blind trial of prostaglandin E₂ gel for cervical ripening and meta-analysis

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The ability of prostaglandin E_2 to prepare the unripe cervix before an indicated labor induction is controversial. We therefore tested 100 pregnant women in a randomized, double-blind trial with intracervical prostaglandin E_2 gel, 0.5 mg. The 53 women who received the placebo gel had an outcome similar to that of the 47 women who received the prostaglandin E_2 gel. The mean change in cervical score, mean application-to-delivery interval, incidence of uncomplicated spontaneous labor, incidence of cesarean delivery for a failed induction, and the overall cesarean section rate were not significantly different for the two groups. A meta-analysis, incorporating 18 studies of 1811 patients who received a single application of at least 5 mg prostaglandin E_2 gel intravaginally or 0.5 mg intracervically, demonstrated no significant decrease in the overall cesarean delivery rate (p=0.85). We conclude that the use of single-dose intracervical prostaglandin E_2 gel for cervical ripening has little effect on labor induction. Moreover, the use of single-dose intracervical or intravaginal prostaglandin E_2 gel does not alter the incidence of cesarean delivery, even when large numbers of patients are analyzed by combining the results of similar reports. (AM J OBSTET GYNECOL 1991;165:991-6.)

Key words: Prostaglandin E2, cervical ripening, metaanalysis

The dilatation, effacement, and other components of the cervical score have a significant effect on the success of an indicated induction of labor. Recently, prostaglandin E₂ (PGE₂) gel has been shown to increase the cervical score and facilitate an oxytocin labor in-

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Reprint requests: John Owen, MD, Department of Obstetrics and Gynecology, University Station, Birmingham, AL 35294. 6/6/31431 duction.²⁻⁷ Whether the use of PGE₂ gel actually improves the cesarean delivery rate is unclear. The purpose of this study was to evaluate the efficacy of a single dose of 0.5 mg of PGE₂ gel for cervical priming before an indicated induction of labor. Furthermore, we have used the technique of meta-analysis to evaluate the role of PGE₂ gel in obstetrics by assessing its influence on the cesarean delivery rate.

Material and methods

Randomized, double-blind study. From March 1, 1987, to Nov. 30, 1989, we obtained informed consent to test PGE₂ gel as a cervical ripening agent. The study

was approved by the institutional review board at the University of Alabama at Birmingham. Candidates were recruited from among patients who were receiving obstetric care in the local health departments or in the University of Alabama at Birmingham obstetric complications clinic. Women with a maternal or fetal indication for labor induction,⁸ a Bishop score ≤4, no evidence of intractable fetal distress, and intact membranes were eligible for the study. Women with known hypersensitivity to prostaglandins, history of asthma or glaucoma, or vaginal bleeding were excluded. Patients who had failure of a previous attempt at labor induction, who had a contraindication to labor induction,⁸ or who were having spontaneous contractions (six or more per hour) also were excluded.

All study patients were admitted to the labor suite where electronic fetal monitoring and tocodynamometry were initiated and venous access was obtained. After a digital examination was performed to determine the preapplication Bishop score,8 a speculum was used to visualize the cervix, and 1 ml of gel was inserted into the cervical canal with a small, flexible catheter. Each patient remained recumbent for at least 1 hour while fetal heart rate and uterine activity monitoring were continued. Thereafter, patients were permitted to ambulate for brief periods, but external monitoring was otherwise continued throughout the gel latent period. After 12 hours a repeat cervical examination was performed to determine the postapplication Bishop score. If contractions sufficient to cause progressive cervical change ensued, labor was diagnosed and the postgel cervical scoring was omitted. Similarly, if the fetal heart rate tracing became nonreassuring, necessitating placement of internal monitoring devices, or if another significant obstetric intervention occurred during the latent period, a repeat cervical score also was not determined. Otherwise, induction of labor was then undertaken with a dilute oxytocin infusion, beginning at 1 mU/min, with escalating doses at intervals of 15 to 30 minutes until a satisfactory uterine contraction pattern (three in 10 minutes) was established. Amniotomy and placement of internal monitoring devices were generally postponed until progressive cervical change was documented. Once begun, the oxytocin infusion was generally continued for a minimum of 12 hours before a failed induction was diagnosed and a cesarean delivery performed. However, serial inductions were permitted, and the decision to extend the induction to subsequent days was made by the attending faculty. In these cases the oxytocin induction was suspended overnight. The diagnosis of a failed induction of labor and the decision to perform a cesarean delivery for this indication were made by the attending faculty.

The active gel was prepared in the hospital pharmacy by slowly melting a 20 mg PGE_2 suppository (Upjohn) and suspending it in hydroxyethylcellulose gel (K-Y jelly, Johnson & Johnson), yielding a final PGE_2 concentration of 0.5 mg/ml.⁹ The placebo gel consisted only of hydroxyethylcellulose, and 1 ml of each mixture was placed in a syringe and catheter. Syringes containing the active gel were stored at -4° C until use, at which time the samples were gradually warmed to room temperature, brought to the labor suite, and administered. Active products were reformulated as necessary with a maximum shelf-life of 35 days. Randomization was performed in the pharmacy and directed by the contents of the next sealed envelope.

Data were analyzed using χ^2 , Fisher's exact test, and the Student t test where appropriate. A p value ≤ 0.05 defined statistical significance.

Meta-analysis. A search of the English language literature from 1966 through 1989 was performed to identify reports of PGE₂ applied locally for cervical ripening before induction of labor. Minimum inclusion criteria were use of a randomized, controlled (either a placebo or no-treatment group) study design, a living fetus, an unfavorable cervical score, an intact amnion, a single local application of PGE₂ gel into the vagina or cervical canal, a minimum dose of 0.5 mg intracervical or 5 mg intravaginal, subsequent labor induction with a continuous dilute oxytocin infusion, and an interpretable cesarean delivery rate. Failure to use a doubleblind design or even an unblinded application of placebo gel did not prevent inclusion in this analysis as long as an untreated, randomized control group was included. Control groups that were actually treated by immediate amniotomy or with a continuous oxytocin infusion were excluded. All reports were identified by using a computerized MEDLINE search (performed by two independent, experienced researchers), a bibliography review of identified articles, the proceedings of the Society of Gynecologic Investigation, 1984 to 1990, the proceedings of the Society of Perinatal Obstetricians, 1984 to 1990, and a query to the Oxford Database of Perinatal Trials.¹⁰ Reports whose titles clearly implied that the PGE2 was being used for an induction of labor (as opposed to cervical ripening) or that a route of administration other than locally applied PGE₂ gel was used were not examined. All other reports were considered for potential inclusion, and either the abstract or the entire article was examined with reference to the inclusion criteria. If a particular report could not be excluded solely on the basis of its abstract (e.g., lower or multiple doses were used), then the entire article was reviewed before a final decision was made. Fisher's χ^2 method¹¹ for combining results was used to conduct the meta-analysis.

Table I. Indications for labor induction

Indication	Placebo $(n = 53)$	$PGE_2 \ gel \ (n = 47)$	p Value
Oligohydramnios	21	11	0.08
Postterm pregnancy	5	7	0.40
Hypertension	11	13	0.42
Diabetes mellitus	7	6	0.95
Abnormal results of fetal testing	5	3	0.72
Suspected fetal growth restriction	1	2	0.60
Other	3	5	0.47

Results

Randomized, double-blind study. One hundred patients were enrolled in the study (53 received the placebo and 47 the active gel). Indications for induction of labor are shown in Table I and were similar between the two groups. There was no significant difference between the two groups with regard to race, maternal age, gestational age at the time of delivery, preapplication Bishop score, or mean birth weight (Table II). Selected pregnancy outcome parameters are listed in Table III.

During the 12-hour latent phase, uncomplicated spontaneous labor occurred in six patients, three in each group. Additionally, in two patients in the placebo group spontaneous contractions developed and a nonreassuring fetal heart rate tracing necessitated amniotomy and placement of internal monitors. Conversely, in the PGE2 group eight patients required a significant obstetric intervention during the latent period (p = 0.04). Five underwent amniotomy for fetal heart rate abnormalities after the onset of spontaneous contractions. Two patients required a cesarean delivery during the latent period, one for persistent late decelerations with spontaneous contractions and the other for worsening maternal hypertension. In one additional patient in the active gel group hyperstimulation developed but responded to vaginal irrigation and parenteral magnesium sulfate. Omitting these 16 patients (five from the placebo group and 11 from the active group), the mean change in cervical score during the latent period was 1.0 if the placebo was administered and 1.5 if the active gel was used (p = 0.16). The mean interval from placement of the gel to delivery was 28 hours in the women who received the PGE2 versus 34 hours in those who received the placebo (p = 0.08). However, two patients in the control group were omitted from the analysis; both patients received what was deemed to be an adequate trial of oxytocin induction by their attending physician but in neither case did the maternal-fetal condition warrant a cesarean delivery because of failed induction. Each patient was discharged and later readmitted in spontaneous labor. One underwent a cesarean delivery because of a labor arrest, and the other was delivered vaginally.

Table II. Population characteristics

	$Placebo \\ (N = 53)$	$PGE_2 \ gel$ $(N = 47)$	p Value
Caucasian (%)	36	36	0.97
Maternal age (yr)	23.7	24.4	0.57
Gestational age (wk)	38.5	39.1	0.31
Nulliparous (%)	62	47	0.08
Initial Bishop score	2.8	2.4	0.08
Birth weight (gm)	3142	3148	0.96

Considering the events documented here during the 12-hour latent phase and those that were observed during the subsequent induction of labor, the overall cesarean section rate in the women who received the PGE2 was 28% (13/47) as compared with 30% (16/53) in those who received the placebo (p = 0.78). There was no difference between the two groups with regard to the indications for cesarean section. Failed labor induction, dystocia, and fetal distress occurred with statistically similar frequencies. One patient in the placebo group was found to have an unsuspected breech presentation at the end of the latent period, whereas two other patients in the PGE2 group were delivered abdominally because of amnionitis and worsening maternal hypertension, respectively (as noted). All of the 100 neonates had a 5-minute Apgar score of ≥7 whereas four neonates in each group had an umbilical arterial pH < 7.2.

Complications related to the placement of the active gel were infrequent. One patient who received the PGE₂ experienced uterine hyperstimulation and fetal bradycardia, as noted above. After its resolution the patient received oxytocin and was delivered vaginally. In another patient hyperstimulation also developed; management was expectant, and she was delivered vaginally. One patient had nausea and vomiting. No patients experienced diarrhea, significant hypotension, or fever during the latent period.

Meta-analysis. A total of 18 reports that met our inclusion criteria were identified. These are listed with

Table III. Pregnancy outcome,

,	Placebo (n =	= 53)	$PGE_2 \ gel'(n = 47)$	p Value
Gel latent period				
Uneventful spontaneous labor	3	٠, .	3	1.0
Iatrogenic interventions	2		8	0.04
Mean Bishop score change*	1.0	•	1.5	0.16
Mean gel-to-delivery time† (ḥr)	34 '		28	0.08
Cesarean delivery	16 (30%)		13 (28%)	0.78
Failed induction	4	. "	2	0.68
Fetal distress	6		. 4	0.75
Arrest of labor	. 5	•	5	1.0
Other	1 /		2	
Mean umbilical artery pH	,7.25		7.24	0.38

^{*}Only patients with an uneventful gel latent period; see text.

Table IV. Randomized, controlled trials of PGE2 gel

			Dose				N		esarean ction	FET
Author	Year	Source	(mg)	Route	Placebo	PGE_2	Control	PGE_2	Control	(p)
Berstein	1986	AM J OBSTET GYNECOL 156:335	0:5	Ċ	+	52 [°]	52	31 .	25	0.66
Hutchon	1980	Int J Gynecol Obstet 17:604	0.65	C .	. +	18	· 35	44	37	0.77
Kristofferson `	1986	Int J Gynecol Obstet 24:297	0.5	C.	_	25	25	16 .	12 · ،	1.00
Laube	1986	Obstet Gynecol 68:54	0.5	C	+	20	20	15	5	0.61
MacKenzie	1979	Br J Obstet Gynaecol 86:167	5.0	V	+	16	. 16	6	19	0.60
Medearis	1990	SPO Abstract No. 13	0.5	· · C ·	+	174	.: 91	22	18	0.52
Nager	1987	J Perinatol 7:189	0.5	С	_	19.	15	26	47	0.29
Nimrod	1984	Obstet Gynecol 64:476	0.5	С.	+	15 .	15	13	0	0.48
Noah	1987	Acta Scand 66:3	0.5	C ',	`	416	404	16	21	0.09
Owen	1991	AM J OBSTET GYNECOL 164:313	0.5	, C .	+	47	53	27	30 -	0.83
Prins	1983	Obstet Gynecol 61:459	5.0	V ·	+	15	15	27	47	0.45
Trofatter	1985	AM J OBSTET GYNECOL 153:268	0.5	C	*	30	29	27	31	0.78
.Ulmsten	1985	Arch Gynecol 236:243	0.5	Ċ	+	19 -	20	16	30	0.45
Williams	1985	Obstet Gynecol 66:769	5.0	V	+	20	. 20	35	35	1.00
Wingerup	1978	Acta Scand 57:403	1.0	C	+	10	10	0 .	30	0.21
Wiqvist	1986	Acta Scand 65:485	0.5	. С	.—	25	25	28	56	0.08
Yonekura	1985	SPO Abstract No. 90	0.5	С.	_	16	14	38	28	0.71
Zuidema	1985	SPO Abstract No. 210	0.5	C		7	. 8	43	63	0.62

FET, Fisher's exact test, two-tailed; C, intracervical; V, intravaginal; SPO, Society of Perinatal Obstetricians.

selected descriptive data in Table IV. These reports included 944 treated and 867 control patients. The p value obtained from Fisher's χ^2 method was 0.85, and therefore the null hypothesis could not be rejected at the 0.05 level.

Comment

The state of the cervix is clearly related to the success of a labor induction. As Bishop¹ reported in 1964, when the cervical score exceeded 8, the incidence of vaginal delivery after labor induction was not significantly lower than that observed after spontaneous labor. Current guidelines of the American College of Obstetricians and Gynecologists state that a cervical score of at least six is considered favorable and is more likely to result in a successful labor induction. However, while the process of natural cervical ripening predicts a suc-

cessful labor induction, the effects of iatrogenic ripening are less well defined and remain under clinical investigation.

The results of our randomized study did not confirm that the use of PGE₂ gel for cervical ripening improves the success rate of an indicated labor induction. We found no significant difference in the postapplication Bishop score, the incidence of uncomplicated spontaneous labor after placement of the gel, the application-to-delivery interval, the overall cesarean section rate, and the failed induction rate. With respect to the observed gel-to-delivery time, the exclusion of two patients in the placebo group naturally affected the analysis. Conversely, significantly more patients in the PGE₂ group required a significant obstetric intervention during the gel latent period, which also may have influenced the results. Furthermore, because the placebo

[†]Excludes two control patients; see text.

^{*}Sham insertion.

group actually experienced a 12-hour idle period without a uterotonic agent, it is plausible that the immediate institution of the oxytocin infusion would have significantly shortened their time to delivery. Therefore we view the interpretation of this outcome parameter in this and other reports as problematic. Finally the inclusion of more nulliparous patients in the placebo group (p = 0.08) should have served to magnify the beneficial effects of the active gel.

We chose the total cesarean rate as the sole outcome parameter in the meta-analysis because it is the most clearly defined and unbiased end-point of a labor induction. The primary reason to perform a labor induction is to prevent a cesarean delivery; therefore any new technique advanced to modify the induction procedure should be critically examined against its influence on the cesarean delivery rate. We included only series that were based on a randomized, controlled study design because of its immunity to various biases. We limited the investigation to reports that used at least 5 mg intravaginally or 0.5 mg intracervically because, if PGE2 made a significant impact on the cesarean rate, it likely would be most notable with the higher doses. Reasonably, if the maximum doses were ineffective, lesser ones would not be expected to be more efficacious. Last, we selected only reports that used a viscous gel medium, since this kind of homogeneous mixture presumably allows controlled absorption of the PGE₂. 12 Reports that were based on the use of PGE₂ in other carriers also have appeared but were not considered because of the relative paucity of available clinical information concerning these methods.

Major limitations of meta-analysis include (1) the difficulty of locating reports that have a similar study design and that evaluate the same outcome parameters and (2) ensuring that all available reports on the subject have been considered. Nevertheless, by performing a computerized literature search, a bibliography review, a search for peer-review data presented at our two primary obstetric national meetings, and a query to the Oxford Database of Perinatal Trials, we believe that we have included the vast majority of reports that met our inclusion criteria. Still, the problem of unpublished data persists.13 We determined that it would require at least seven additional reports, each with a p value of 0.05, or 11 reports, each with a p value of 0.10, to reverse the findings of this meta-analysis. Most reports of PGE2 gel for cervical ripening have shown some benefit from its use, but the benefit most commonly has been a significant change in the cervical score. 5-6 However, this has not been a universal finding.2,7 Other clinically useful parameters, such as the time to delivery or the failed induction rate, are not universally reported and also may suffer from biases that are secondary to labor management strategies or other, sometimes arbitrary decisions by attending physicians. Whereas the incidence of serious systemic side effects from the PGE2 gel appear to be low, its tendency to induce spontaneous (sometimes hyperstimulatory) contractions^{3-6, 14} may lead to fetal compromise. If recognized, this generally can be treated with tocolytic agents or emergency delivery. Nevertheless, the potential for uterine hyperstimulation makes PGE2 gel less than the ideal cervical ripening agent. Compared with PGE2, other cervical ripening methods have been shown, in relatively small series, to have similar efficacy with less cost and fewer side effects. These include the use of mechanical dilators, 15 oxytocin infusion, 16 and extraamniotic saline solution.14 There may be an editorial bias that favors the publication of studies that demonstrate a benefit from a particular therapy¹⁷; therefore we believe that our inability to show a particular benefit (a difference in the cesarean delivery rate) from the use of PGE2 gel supports the concept that there are few unreported, positive studies that would reverse our findings.

In summary, in spite of earlier reports on the efficacy of locally applied PGE2 for cervical ripening, our data and a meta-analysis do not confirm that this technique can improve the success of an indicated induction of labor by significantly decreasing the cesarean delivery rate. Given that the use of this pharmacologic agent is not without side effects, we cannot recommend PGE2 gel for cervical priming before an indicated induction of labor.

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Uterine rupture during trial of labor after previous cesarean section

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This study was undertaken to determine the incidence and associations of uterine rupture and dehiscence with an attempted vaginal birth after cesarean section. The charts from 137 patients who had uterine scar separation after a previous cesarean section from 1983 to 1989 were examined. Approximately 9.3% of the 119,395 women who were delivered in that interval had a prior cesarean section. Of those, 68.8% underwent a trial of labor with a 79.2% success rate. The uterine rupture rate in this latter group was 0.8%, while an additional 0.7% had a bloodless dehiscence. Bleeding and pain were unlikely findings with a uterine scar separation (3.4% and 7.6%, respectively). The most common manifestation of a scar separation was a prolonged fetal heart rate deceleration leading to operative intervention (70.3%). We conclude that, although the incidence of uterine rupture was low, the event is most often seen as an acute emergency. Prevention should be directed toward timely diagnosis and prompt management of labor dystocias. Staff and facilities for safe management of a uterine scar separation are a requisite for the conduct of a vaginal birth after previous cesarean section. (AM J OBSTET GYNECOL 1991;165:996-1001.)

Key words: Uterine rupture, cesarean section, dehiscence

The incidence of cesarean section in the United States remains at a high level, approximately 25%. Financial and medical considerations and patient preferences have initiated pressures to decrease the number of cesarean sections by promoting vaginal birth after a

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previous cesarean section. Counter balancing this trend is concern about a possible catastrophic scar separation during labor. Previous studies have estimated the incidence of uterine scar separation to be between 0.2% and 3.5%. 2-6 However, interpretation of incidence figures has been complicated by a lack of agreement on definitions. Uterine rupture has not been uniformly distinguished from uterine scar dehiscence. Bloodless uterine scar dehiscence has not been shown to have serious consequences for the mother or infant, whereas the effect of uterine rupture can be devastating to both.

Previous studies from this department and others⁷⁻¹⁰ have demonstrated the relative safety of a trial of labor after a cesarean section. The low incidence and small numbers of uterine ruptures, however, have precluded a comprehensive examination of this complication.

		Dehiscence		Rupture	
	n	%	No.	%·	No.
All patients with previous cesarean section	11,041	0.6	67	0.6	70
Elective repeat cesarean section	3,443	0.4	13	0.3	9
All patients with trial of labor	7,598	0.7	54	0.8	61
Successful trial of labor	6,021	0.5	28	0.1	7
Failed trial of labor (ce- sarean section)	1,577	1.7	26	3.4	54

Table I. Occurrence of scar separation in patients with previous cesarean section

This study was undertaken to examine the Los Angeles County-University of Southern California Women's Hospital experience with uterine rupture in patients who underwent a trial of labor after previous cesarean section. An attempt was made to define maternal risk factors associated with a uterine rupture.

Material and methods

The study population consisted of patients who were delivered at the Los Angeles County-University of Southern California Women's Hospital, the teaching hospital of the University of Southern California School of Medicine. Patients with a previous cesarean section who had a scar separation were identified during the period from January 1983 through December 1989 from coded medical records and department morbidity and mortality review documents. The year 1983 marked the beginning of the systematic introduction of vaginal birth after previous cesarean section. The records of 137 patients with a scar separation were retrospectively reviewed.

The patients who underwent a trial of labor had either a low-transverse, low-vertical, or unknown uterine incision. Exclusion criteria from 1983 through 1985 were an unknown uterine scar, a previous classic incision, or transverse lie that failed version. After that time, patients with unknown scars were included. Patients with multiple gestation or breech presentation were not excluded. If the patient refused a trial of labor, a repeat cesarean section was planned.

The fetal heart rate was monitored with a fetal scalp electrode or external Doppler technique. Uterine activity was monitored with an intrauterine pressure catheter when cervical dilatation allowed or with an external tocodynamometer. In each case blood was available and intravenous access established. Oxytocin and an epidural anesthetic were administered for obstetric indications. A uterine exploration was performed after each successful vaginal delivery to establish scar status. Patient management was conducted by resident physicians with staff supervision.

Uterine rupture was defined for our purposes by a modification of Donnelly and Franzoni.11 Uterine dehiscence was defined as a uterine wall defect with no emergent laparotomy, no evidence of fetal distress, and no excessive bleeding. A uterine rupture was defined as a uterine wall defect and emergency laparotomy, acute fetal distress necessitating operative intervention, or acute maternal bleeding with an estimated blood loss of >1 L.

Statistical analysis was conducted with the Crunch statistical software package (Crunch Software Corp., Oakland, Calif.). A t test was used to compare groups of patients, with p < 0.05 considered significant. Timetrend analysis was conducted with the BMDP package (BMDP Statistical Software Inc., Los Angeles) statistical software with a Cochran-Mantel-Haenszel analysis.

Results

During the period from January 1983 through December 1989 there were 119,395 deliveries at Los Angeles County-University of Southern California Women's Hospital, including 11,041 (9.3%) women with a history of a cesarean section. Of these, 7598 patients (69%) underwent a trial of labor, with 6021 (79.2%) delivered vaginally. The uterine rupture rate with a previous cesarean section was 0.6% (70 cases), whereas the uterine dehiscence rate was 0.6% (67 cases). The distribution of patients into trial of labor and elective repeat cesarean section groups is shown in Table I. In those patients who underwent a trial of labor, the incidence of uterine rupture and dehiscence was 0.8% (61 cases) and 0.7% (54 cases), respectively. The uterine rupture and dehiscence rates over the years studied are shown in Table II for patients who underwent a trial of labor.

Fetal distress was the primary indication for cesarean section in patients with a uterine rupture (85.2%, 46/54 cases), with arrest disorders second (13.0%, 7/54 cases). In patients with a uterine dehiscence, arrest disorders were the most common indication for cesarean section (69.2%, 19/26 cases), with fetal distress sec-

Table II. Incidence of scar separation in patients who underwent trial of labor after previous cesarean section, by year

		Dehiscence		Rupture	
Year	n	%	No.	%	. No.
1983	908	0.1	1	0.4	4
1984	1153	0.8	9	0.4	5
1985	1367	0.4	. 6	0.6	7
1986	1131	1.3	15	1.0	11
1987	730	0.8	6	1.2	10
1988	999	0.6	6	0.4	4
1989	1310	0.8	11	1.5	20
TOTAL	7595	0.7	(54)	0.8	(61)

Cochran-Mantel-Haenszel trend analysis; statistically significant increase in rate of uterine rupture (p = 0.003) with no significant increase in rate of uterine dehiscence.

Table III. Occurrence of uterine scar separation as function of type of previous uterine incision in patients who underwent trend of labor

Type of previous uterine incision	Dehiscence	e (n = 54)	Rupture $(n = 61)$	
	No.	%	No.	%
Low-transverse	37	68.5	29	. 47.5
Unknown	16	29.6	25	41.0
Classic	0	0	5	8.2
Vertical	1 .	1.9	2	3.3

ond (26.9%, 7/26 cases). The seven cases of fetal distress in the uterine dehiscence group were not believed to be related to the scar separation, because the dehiscences were small (<2 cm).

The type of previous uterine incision in patients who underwent a trial of labor, either low-transverse, low-vertical, classic, or unknown, is shown in Table III. The type of previous scar was identified from operative reports or by intraoperative identification of the scar site. Eleven of 115 patients (9.5%) who underwent a trial of labor had uterine rupture that was distant and separate from the previous scar.

The average number of previous cesarean sections in those patients who underwent a trial of labor was 1.4, with only four patients having more than two previous cesarean sections. Patients were not excluded from a trial of labor on the basis of the number of previous cesarean sections, nor was the indication for previous cesarean section a cause for exclusion, with the exception of a previous scar separation. In such patients a repeat elective cesarean section was recommended. The number of successful vaginal deliveries after a cesarean section is shown in Table I for the trial-of-labor population.

Oxytocin was used during the trial of labor in 73.8% of the patients with a uterine rupture and in 74.1% of the patients with a uterine dehiscence. Overall use of oxytocin during a trial of labor was 74.0% of patients

with a scar separation. An epidural anesthetic was administered during labor to 27.9% and 35.2% of the uterine rupture and dehiscence populations, respectively. The concurrent administration of epidural anesthesia and oxytocin was seen in 24.6% of the uterine rupture and 33.3% of the uterine dehiscence groups. Average labor duration in those patients with a uterine rupture was 14.8 ± 10.6 hours, whereas the average duration was 15.0 ± 8.2 hours in patients with a uterine dehiscence.

The maternal and infant variables of age, gravidity, parity, estimated blood loss, Apgar scores, birth weight, and gestational age are shown in Table IV for patients with a scar separation. Maternal morbidity and mortality as evidenced by maternal death, transfusion, urologic injury, and hysterectomy are seen in Table V. Thirteen patients were transfused, with an average of 2.7 units per patient. No patient with uterine dehiscence received a transfusion. The 15 urologic injuries consisted predominantly of intentional cystotomy or bladder laceration, with no ureteral injuries reported. Cystotomy with or without the passage of stents was most often done for the determination of ureter position or patency. Fourteen hysterectomies were performed in the uterine rupture group. Neonatal morbidity and mortality, as evidenced by neonatal death, fetal death, and neonatal intensive care unit admission, are also seen in Table V. There were no late infant

Table IV. Patient characteristics

	Dehiscence $(n = 67)$	Rupture (n = 77)
Age (yr)	27.9 ± 4.7	28.1 ± 5.5
Gravidity	3.8 ± 1.8	3.6 ± 1.7
Parity	2.1 ± 1.3	2.0 ± 1.2
Estimated blood loss (ml)	550 ± 300	1500 ± 1000
Apgar score at 1 min < 7 (%)	27.8	52:5
Apgar score at 5 min <7 (%)	5.6	27.8
Birth weight (gm)	3450 ± 600	3500 ± 500
Gestational age (wk)	40.0 ± 2.1	40.0 ± 1.8
Duration of labor (hr)	14.8 ± 10.6	15.0 ± 8.2

Values are mean ± 1 SD. Apgar scores are reported as the percentage of patients with Apgar scores <7.

deaths (>28 days) in any of the scar separation infants. All infants admitted to the neonatal intensive care unit left the hospital with the mother.

Fetal death was associated with uterine rupture and extrusion into the abdominal cavity of a 2680 gm, 40week fetus. The infant was intubated with full cardiopulmonary resuscitation for 20 minutes without success. The autopsy results were normal with the exception of petechial pulmonary hemorrhage and lack of lung aeration.

Death occurred in a 1540 gm female fetus delivered at 39 weeks. The patient was seen in labor with fetal death documented by ultrasonography. A macerated, otherwise normal infant with an autopsy-based gestational age of 32 weeks was delivered after a 7-hour labor. Uterine exploration after vaginal delivery revealed a 2 cm uterine scar separation along a previous low-transverse incision that was not repaired.

The maternal death involved a 37-year-old, gravida 3, para 1 woman who arrived with a 40-week intrauterine pregnancy and spontaneous rupture of membranes. Her obstetric history was remarkable for a cesarean birth with her last pregnancy 6 years earlier in El Salvador, performed because of failure to progress in labor. Labor was augmented with oxytocin, resulting in a 13-hour first stage of labor. The second stage of labor was 3 hours long, with delivery of a 3700 gm infant (Apgar scores of 3 and 8) facilitated by Tucker-McLane forceps. The uterus was manually explored in the delivery room, with no scar separation detected. Approximately 2.5 hours after delivery the patient had a cardiopulmonary arrest secondary to intraabdominal hemorrhage. Laparotomy disclosed a left lateral uterine rupture (approximately 10 cm) disrupting the left uterine artery; a supracervical hysterectomy was subsequently performed. Estimated blood loss was 5 L. Although the patient's initial condition was stable, there was subsequent deterioration with evidence of septic shock, consumptive coagulopathy, and extensive bronchopneumonia. She died on postpartum day 14.

The number of scar separations that were repaired

Table V. Maternal and perinatal morbidity and mortality associated with uterine scar separation in patients who underwent trial of labor

	$\begin{array}{c} Dehiscence \\ (n = 54) \end{array}$	Rupture (n = 61)
Maternal death	0	1
Transfusion	0	13 (2.7 units/patient)
Urologic injury	2	15
Hysterectomy	0	14
Perinatal death	1	1
Neonatal intensive care unit admission	1	10

Table VI. Incidence of scar separation repair in patients who underwent trial of labor

	Successful vaginal delivery	Cesarean section	Total
Dehiscence	0/28	26/26	26/54
Rupture	2/7	43/54	45/61
Hysterectomy	3/7	11/54	14/115

in patients who underwent a trial of labor is shown in Table VI. Patients who underwent elective cesarean sections all had the scar separation repaired. In trialof-labor patients who underwent a cesarean section, all scars were repaired unless it became apparent that a hysterectomy was indicated because of the extent of the scar separation or bleeding (14 of 80 patients underwent hysterectomy). In the patients who had a vaginal delivery, the scar was not repaired unless there was significant bleeding, in which case a laparotomy was performed for repair or for hysterectomy.

Subsequent pregnancies in the scar separation group were uncommon. Thirteen women were known to have had a subsequent delivery. Three of the 13 had dehiscences that were not repaired; 10 had scar separation that was repaired. The recommendation at Los Angeles County-University of Southern California Women's 1000 Farmer et al.

Table VII. Subsequent pregnancy outcome in patients with uterine scar separation

	Intact scars in subsequent pregnancy				
Pregnancy history	Cesarean section	Vaginal delivery	Total		
Dehiscence	5/6	0/1	5/7		
Rupture	4/5	1/1	5/6		

Hospital was, and is, that patients with a scar separation, repaired or unrepaired, should undergo elective cesarean section for subsequent pregnancies. The outcomes for the 13 pregnancies are shown in Table VII. It is of interest that two patients subsequently had an inadvertent trial of labor after a scar separation. One demonstrated a uterine dehiscence on uterine exploration after vaginal delivery with no further complications. The other patient had a vaginal delivery with no scar separation found on uterine exploration.

Comment

The incidence of cesarean section has been increasing for the past several decades. Patient, financial, and medical pressure to decrease this rate have led to the increased use of a trial of labor after a cesarean section. This will doubtless increase the clinician's exposure to the complications of uterine rupture and dehiscence.

The incidence of uterine rupture when the denominator is the total number of patients with a previous cesarean section is comparable to that found by other authors.³⁻¹⁰ The incidence of uterine rupture in patients undergoing a trial of labor is similar to the incidence of 0.6% derived by metaanalysis by Horowitz et al.12 but higher that found by Flamm et al.16 Overall, there is a statistically significant trend (p = 0.003) toward an increasing incidence of uterine rupture over the years of this study when the data are analyzed by the Cochran-Mantel-Haenszel method. Examination of labor duration, deviation from the labor curve, incidence of amnionitis, incidence of oxytocin use, and the number of previous cesarean sections in the population showed no statistically significant changes over the 7 years evaluated in this study. We do not have an explanation at this time for the increasing incidence of uterine rupture.

The finding of an equal incidence of uterine dehiscence and rupture is at variance with the study of Meehan et al.,18 where an approximately 2:1 ratio of dehiscence to rupture was found. The possible explanations for this discrepancy are that this study was larger in numbers and represents a more accurate estimation of the true uterine dehiscence rate or that the presence of uterine dehiscences was not as effectively appreciated by the large number of house staff examinees

participating in this study. Indeed, the maternal death involved a patient who underwent a manual uterine exploration, with a large uterine rupture not appreciated.

Fetal distress in labor as the most common indication for operative intervention in the uterine rupture group affirms observations of Meehan et al. 18 and emphasizes the need for close supervision and monitoring of these patients in labor. However, since there was only one neonatal death attributable to uterine rupture, with otherwise good perinatal outcomes, this does not support the view of Uppington,14 that fetal distress is a "late and sinister" occurrence. Rather, the findings of acute prolonged deceleration as the predominant evidence of fetal distress points to an acute event that, with prompt surgical intervention, resulted in a generally good long-term perinatal outcome. The traditional hallmarks and symptoms of scar separation, abdominal pain and bleeding, were found to occur relatively infrequently, in 3.4% and 7.6%, respectively.

Review of Tables III and IV allows an assessment of the risk factors for uterine scar separation. The type of uterine incision was different in patients with uterine rupture compared with those with a dehiscence. A known low-transverse incision predominated in the dehiscence group, and the unknown scar was more common in the uterine rupture group. A possible explanation for this might be that there was a higher incidence of previous classic cesarean sections in those patients who had a uterine rupture with an unknown scar. Many of our patients come from areas of the world where low-transverse incisions are not as common as in the United States.

Patient parity was not as high as found by other authors,18 with no difference in parity between dehiscence and rupture patients. There were no other statistically significant differences between the patients with uterine rupture and dehiscence with respect to age, gravidity, percentage of infants with a 1-minute Apgar score <7, gestational age, or birth weight. The percentage of infants with a 5-minute Apgar score < 7 was statistically greater in the uterine rupture group (p < 0.003). Whereas the patient may have had one or two previous cesarean sections, the trial of labor was overwhelmingly the first attempt for the patient. The duration of labor in the patients with a uterine rupture was not significantly different than for patients with a dehiscence, nor was the incidence of amnionitis different for the two groups.

Oxytocin use during a trial of labor remains controversial. The use of oxytocin during labor was common in patients with a uterine scar separation (74.0% overall). Since the incidence of oxytocin use during a trial of labor in patients without a scar separation is not available, no odds ratio can be calculated. Similarly, the risk of the combined use of oxytocin and regional anesthesia, thought to be a risk factor¹³ in uterine scar separation, cannot be evaluated from our data. It is possible, in some of these cases, that oxytocin was being used to stimulate a dysfunctional labor secondary to an existent scar separation. However, we believe the benefits of oxytocin use and regional anesthesia outweigh the risks when the informed patient is monitored appropriately.

The policy of this institution has been not to repair uterine dehiscences in the abscence of excessive bleeding. The short-term adverse consequences support this policy, with invariable good maternal outcome in patients so managed. However, the long-term outcome remains unclear. Only three patients with a known unrepaired uterine dehiscence have been identified as having subsequently delivered at this hospital. Two of the three patients had a repeat cesarean section, with no scar defect noted. One patient with a scar dehiscence had a subsequent inadvertent trial of labor with a resultant uterine rupture and hysterectomy. Of the 10 patients with the scar separation repaired, two had a scar separation in the subsequent pregnancy. Of interest, one patient with a repaired scar separation had a subsequent inadvertent trial of labor, with no scar separation detected on uterine exploration. The policy at this institution is also to advise patients with a previous scar separation to undergo a repeat cesarean section with the next pregnancy. In nine of 11 patients who followed this protocol, there was no evidence of scar separation at the time of the repeat cesarean section.

In conclusion, the risk of a uterine scar separation is small but finite. A trial of labor in a patient with a previous cesarean section may be conducted with minimal sequelae when the patient is continuously monitored and facilities for an emergency delivery are met. Surgical expertise and blood-banking and anesthesia services, with available urologic consultation, are mandatory to deal with the occasional patient who encounters life-threatening complications associated with a vaginal birth after a previous cesarean section.

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Evaluation of obstetric ultrasonography at the first prenatal visit

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A prospective study was performed on 1000 consecutive patients to assess the value of ultrasonography at first prenatal visit. Significant ultrasonographic findings were noted in more than one third of the patients. A discrepancy between ultrasonography and menstrual dating was the most frequently noted abnormality, occurring in 27%; 59% of those would not have been discovered on initial physical examination. Other frequently noted abnormalities included missed abortion (5.7%) and twins (1.6%). The frequency of abnormalities on the initial laboratory studies recommended by the American College of Obstetricians and Gynecologists was evaluated and found to be significantly less than the frequency of clinically important ultrasonographic findings, ranging from 4.0% for positive urine cultures to 0.2% for positive serologic tests. Unexpected findings on physical examination, excluding assessment of pregnancy dating on the basis of uterine size, were even less frequent, occurring in 2.1% of the patients. It is concluded that obstetric ultrasonography at the time of the first prenatal visit is better than physical examination in evaluating the status of the pregnancy and should be considered for all patients. (AM J OBSTET GYNECOL 1991;165:1002-5.)

Key words: Obstetric ultrasonography, prenatal care, clinical obstetrics

The first prenatal visit is considered to be an extremely important time to evaluate the status of the mother and her pregnancy, including thorough medical history, physical examination, and laboratory evaluation. Although the first prenatal visit would seem to be an excellent time to more extensively assess the status of the pregnancy with ultrasonography and many offices and clinics have ultrasonography units for immediate availability, the routine use of obstetric ultrasonography at the time of the first prenatal visit has been neither fully evaluated nor recommended. This is in spite of evidence from multiple studies that have shown that ultrasonography performed in the first half of pregnancy frequently changed the gestational age as estimated by last menstrual period,1-3 enhanced early detection of twin pregnancy4,5 and missed abortion,6 and facilitated the diagnosis of fetal growth retardation.7

A prospective study was performed to evaluate the role of routine obstetric ultrasonography at the time of the first prenatal visit. Special emphasis in this study was placed on evaluating the ability to diagnose discrepancies between ultrasonography and menstrual

dating by physical examination and also to compare the frequency of clinically pertinent ultrasonographic findings with the frequency of clinically pertinent findings on physical examination and the laboratory studies recommended by the American College of Obstetricians and Gynecologists to be performed routinely at the first prenatal visit.⁸

Material and methods

A prospective study was performed on consecutive private patients presenting for a first prenatal visit. Each patient had a thorough medical history, physical examination, and obstetric ultrasonography performed in that order by a single examiner (J.M.B.). Uterine size was measured by bimanual examination in the first 12 weeks of pregnancy, by abdominal examination from 13 to 20 weeks' gestation, and by tape measure after 20 weeks' gestation. Ultrasonographic studies were performed with an Ultramark 4A real-time unit (Advanced Technical Laboratories, Bothell, Wash.). Abdominal linear-array and sector scanners and an intravaginal transducer were used as indicated for evaluation. Ultrasonographic estimation of gestational age was based on gestational sac size before 6 weeks, crownrump length from 6 to 12 weeks, and after 12 weeks the average of biparietal diameter, head circumference, abdominal circumference, and femur length. The laboratory tests were those recommended in ACOG's "Standards for OB/GYN Services," 1989.8 These tests included hemoglobin, urine culture, blood group, and Rh-type determination, antibody screen, syphilis screen, and cervical cytologic results.

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The parameters for an inconsistency between ultrasonographic dating and menstrual dating were: before 13 weeks, >7 days; 13 to 20 weeks, >10 days; 21 to 26 weeks, >14 days; after 26 weeks, >21 days.

The parameters for an inconsistency between menstrual dating and uterine size were: before 13 weeks, >2 weeks; 13 to 26 weeks, >3 weeks, and beyond 26 weeks, >4 weeks.

Results

A total of 1000 consecutive patients were studied. In this study group 737 patients were in the first trimester, 200 in the second trimester, and 63 in the third trimester. A clinically pertinent ultrasonographic finding occurred in 36.5% (Table I).

In the 869 patients who had a viable pregnancy and who presented with what was thought to be a reliable menstrual history, 26.8% had a discrepancy between ultrasonographic dating and menstrual dating (Table II). In these patients there was believed to be no discrepancy in dating on the basis of comparison of uterine size and menstrual history in 62% in the first trimester, 48% in the second trimester, and 46% in the third trimester (Table III). The discrepancy between dating derived from ultrasonography and menstrual history had the menstrual dates greater than the ultrasonography dates in 84% of patients in the first trimester, 50% in the second trimester, and 62% in the third trimester.

Evidence of missed abortion was noted in 7.6% of all patients presenting in the first trimester. Of these patients 46% had no discordance between uterine size and menstrual dating, and 41% had not experienced bleeding.

The diagnosis of twin gestation was made in 16 patients. Twelve of these 16 uteri were large for dates on examination. Four sets of twins were diagnosed in each of the second and third trimesters, and in each case the uterine size was large for dates.

Gestational trophoblastic disease was diagnosed in three patients. Two of these patients had uterine size consistent with the menstrual dates.

Clinically pertinent abnormalities on physical examination, excluding evaluation of uterine size, were uncommon (Table IV). Of the 365 patients with abnormal ultrasonographic findings, 160 (47.0%) of these abnormalities were suspected clinically. In 25 patients, 2.9% of those with a viable pregnancy and an apparently reliable menstrual history, there was a discrepancy between uterine size and menstrual dating in spite of the fact that ultrasonographic dating was consistent with the menstrual dating. For clinical evaluation of the status of the pregnancy by pelvic examination, the sensitivity was 44%, specificity 94%, predictive value of an abnormal examination 86%, predictive value of a normal examination 72%, and efficiency 75%.

Table I. Significant abnormalities on initial ultrasonography

Abnormality	%
Discrepancy between ultrasonography and menstrual dating	26.8
Missed abortion	5.6
Twins	1.6
Uterine leiomyomas	1.2
Fetal anomalies	0.5
Gestational trophoblastic disease	0.3
Not pregnant	0.3
Ectopic pregnancy	0.1
Suspicious ovarian mass	0.1
TOTAL	36.5

Table II. Results of comparison of ultrasonographic dating with menstrual dating (N = 869)

Ultrasonographic dating	Concordance of ultrasonography and dates	Difference in ultrasonography and dates
Before 13 wk	460	180 (28.1%)
13-26 wk	138	40 (22.5%)
After 26 wk	38	13 (25.5%)
TOTAL	636	233 (26.8%)

Significant laboratory abnormalities ranged from positive urine culture (4.3%) to positive serologic test results (0.2%) (Table V).

Comment

This study evaluates all of the recommended components of the initial prenatal evaluation with the addition of obstetric ultrasonography. All medical histories, physical examinations, and ultrasonographic studies were performed by a single observer, therefore removing the potential for problems related to interobserver variation. In this report clinically pertinent findings on obstetric ultrasonography performed at the time of a first prenatal visit were noted in more than one third of all patients. This was distinctly greater than the frequency of clinically pertinent abnormalities found on physical examination and on the "routine" laboratory testing recommended to be performed at the time of the first prenatal visit. As has been reported in previous studies, ultrasonography had its highest utility in finding discrepancies in gestational age,1-3 assisting with dating the pregnancy, making the early diagnosis of missed abortion,6 and making the early diagnosis of twins.4.5

A discrepancy between menstrual and ultrasonographic dating was the most commonly noted abnormality, occurring in 26.8% of patients. Physical examination, which has been suggested to assist in making the diagnosis of gestational age discrepancy,9 was not

Table III. Frequency of compatibility of menstrual dates and uterine size when menstrual and ultrasonographic dating differed

		Uterine Size and Dates Compatible		
Weeks' gestation by ultrasonography	Menstrual-ultrasonographic discrepancy (days)	No.	%	
<13	8-14 15-21 >21	87/100 17/32 8/48	87 53 17	
Group subtotal		112/180	62	
13-20 Group subtotal	10-17 18-24 >24	12/14 3/8 1/4 16/26	86 38 25 61	
21-26	14-21 22-28 >28	0/2 1/3 2/9	0 33 22	
Group subtotal		3/14	21	
>26	>21 >28	2/3 4/10	67 4 0	
Group subtotal TOTAL		6/13 137/233	46 59	

Table IV. Significant abnormalities on initial physical examination

Abnormality	%
Uterine size incompatible with menstrual dates	16.0
Suspected incompetent cervix	0.6
Abnormal thyroid	0.5
Cervical polyp	0.3
Unexpected cardiac findings	0.3
Suspicious breast mass	0.1
Signs of recent intravenous drug abuse	0.1
Severe scoliosis	0.1
Pelvic mass	0.1

Table V. Abnormal laboratory studies at first visit

Study	%
Positive urine culture	4.3
Not immune to rubella	3.9
Anemia (hemoglobin <10 gm/dl)	2.6
Positive indirect Coombs' test	0.6
Class III or IV Papanicolaou smear	0.4
Positive serologic test result	0.2

found to be particularly beneficial except when wide discrepancies between menstrual dating and ultrason-ographic dating were found. While part of this lack of benefit may be due to the study protocol, it nonetheless shows that physical examination is not precise in dating the pregnancy. While an 8- to 14-day discrepancy between dates and ultrasonography in the first trimester may not seem to be large, in selected cases this difference may be critical. This is especially true in the pregnancy complicated by premature labor, where a 1-week

discrepancy in dating could make a tremendous difference in perinatal management. There is also the obvious importance in making the correct diagnosis of the postterm pregnancy, where routine ultrasonography has been associated with a decrease in the frequency of postterm pregnancy to as low as 1.9%.\text{\text{1}} The finding in this study that most discrepancies in ultrasonographic dating and menstrual dating would postpone the estimated date of confinement as derived from menstrual history is consistent with this decreased likelihood of postterm pregnancy. Accurate pregnancy dating is also extremely important in timing repeat cesarean section, assessing fetal growth, and timing maternal serum α -fetoprotein determination.

In addition to the limitations found in making the diagnosis of a discrepancy in pregnancy dating, other limitations of physical examination assessment of the pregnancy were noted. Uterine size misjudged gestational age in 2.9% of patients where there was no discrepancy between menstrual and ultrasonographic dating and no obvious abnormality of the uterus or pregnancy. Missed abortion was associated with reassuring physical findings in almost half of cases. Only with twins did the physical examination have a high frequency of diagnosis. Because of these limitations, reliance on the first examination to fully assess the status of the pregnancy is believed to be suboptimal.

Other abnormalities found on ultrasonography also were clinically important. The early diagnosis of missed abortion allows timed uterine evacuation when required and also allows the physician to counsel the patient before the onset of bleeding, which we believe facilitates initiation of the grieving process for the pa-

tient. The importance of the early diagnosis of twins, gestational trophoblastic disease, ectopic pregnancy, and suspicious adnexal mass is straightforward.

The relatively low frequency of abnormalities noted on physical examination (except for pelvic examination) and abnormalities noted on the recommended "routine" laboratory studies should not diminish their importance. However, the comparatively high frequency of clinically pertinent findings on first-visit ultrasonography should emphasize the value of this study in initial patient evaluation.

Reasons for caution in performing routine ultrasonography include risks to the fetus, expense, and studies showing no decrease in perinatal morbidity and mortality when ultrasonography is routinely used.9-12 Currently there is no documented evidence of risk of damage to the fetus with the use of diagnostic ultrasonography.18 Evaluation of the expense of the procedure must consider both physician time and monetary costs to the patient. In an office setting where there is an ultrasonographic unit immediately available, these concerns can be addressed more readily than if ultrasonographic capabilities do not exist. In our practice a first-trimester ultrasonography is usually accomplished within 10 minutes. Studies later in pregnancy often take longer, but it is rare to cause an extensive office delay because of the performance of ultrasonography at the first prenatal visit. Because ultrasonography at the first prenatal visit is considered to be an extension of the first physical examination, it is usually performed without charge. If a patient were in a setting where an additional charge for the first prenatal visit ultrasonography is required and if she were in the first trimester, it would probably be more cost-efficient to wait until the second trimester to perform a more detailed screening ultrasonography if routine ultrasonography was to be performed. In our practice, if the first prenatal visit occurs before 16 weeks, follow-up ultrasonography is performed at approximately 20 weeks for further evaluation of the fetal anatomy. Finally, the size and design of this study did not allow assessment of changes in morbidity and mortality related to first prenatal visit ultrasonography.

This study shows that there is a high frequency of clinically pertinent findings on obstetric ultrasonography performed at the first prenatal visit, and it shows the improved accuracy of ultrasonography over physical examination to assess the pregnancy. The conclusion from this study is that the routine use of ultrasonography at the first prenatal visit is valuable and should be considered for all patients.

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Patterns of uterine activity after intravaginal prostaglandin E₂ during preinduction cervical ripening

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In a randomized study, uterine activity patterns were characterized with a portable pressure-sensitive device in 40 nonlaboring women at term with an unfavorable cervix who were undergoing preinduction cervical ripening with prostaglandin E₂. The prostaglandin E₂ was inserted into the posterior vaginal fornix as a single dose of either a 2.5 mg methylcellulose gel (20 cases) or 10 mg controlled-release pessary (20 cases). Uterine activity monitoring began 1 hour before dosing and continued for 12 hours. For those treated with the gel, low-amplitude, high-frequency contractions began within the first hour, reached a peak within 4 hours, and initiated sustained high-amplitude contractions in 10 (50%) cases. With the controlled-release pessary, low-amplitude, high-frequency contractions had a slightly later onset, reached a peak between the fifth and eighth hours, and initiated sustained high-amplitude contractions in 18 (80%) cases. Uterine hyperstimulation occurred in two (10%) pessary cases, with no adverse effect after removal. We conclude that low-amplitude, high-frequency uterine contractions began with either method of intravaginal prostaglandin E₂ delivery but led to sustained, high-amplitude contractions primarily with the pessary. (AM J OBSTET GYNECOL 1991;165:1006-9.)

Key words: Uterine activity, prostaglandin E2, cervical ripening

Worldwide experience has demonstrated that prostaglandin E₂ (PGE₂) administered intravaginally in low doses is of major benefit for preinduction cervical ripening. ^{1, 2} Nearly all patients experience some form of uterine contractions concurrently. Such uterine activity may be desirable if the goal is to ripen the cervix by myometrial contractions, in addition to affecting cervical connective tissues.

Uterine activity measurements made with new ambulatory tocodynamometers correlate well with those made by direct intrauterine and intracervical pressure catheters.³ Interest in monitoring methods arose from observations in patients at risk for preterm birth, in whom uterine activity may not be perceived. In our study uterine activity was characterized by use of sensitive tocodynamometers in pregnant patients at term who underwent preinduction cervical ripening.

Material and methods

After approval was granted by our Institutional Review Board, we enrolled pregnant women at term or beyond (38 to 43 weeks) who required induction of

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labor in spite of an unfavorable cervix (Bishop score ≤4). Indications for cervical ripening in these singleton gestations included postdates, hypertension, diabetes, and suspected fetal growth abnormalities. In all cases average uterine activity recorded beforehand was less than one contraction during a 10-minute window.⁴

As part of a continuing randomized drug trial, the PGE₂ was inserted in the form of either a 2.5 mg gel or a 10 mg controlled-release pessary. The PGE₂ gel was made in our hospital pharmacy by blending a 20 mg PGE₂ suppository with a small amount of methylcellulose gel, then frozen in a 3 ml syringe until needed. The controlled release hydrogel pessary containing a 10 mg reservoir of PGE₂ was provided by the manufacturer (PGE₂ ContRx Infusette, Controlled Therapeutics Ltd., East Kilbride, Scotland). Prior studies have proved the effectiveness of both preparations in causing cervical ripening when placed high into the vaginal canal.^{2, 5}

Each subject received a tocodynamometer (Term-Guard, Tokos Medical Corp., Santa Ana, Calif.) designed for monitoring ambulatory uterine activity. A description of the device and its dimensions, reliability, and mode of operation have been reported previously. This recording and transmission device was used to allow for ambulation without interference. Previous experience has shown the design to be particularly reliable in recording mild contractions at or near term, which predominate during the prelabor period. To ensure optimal measurement of stronger (high-amplitude) uterine contractions, a traditional in-hospital monitor

(model 115, Corometrics, Wallingford, Conn.) was used simultaneously, with the tocodynamometer held in position over the uterus above the umbilicus by an additional belt.

The study research nurse (A.M.M.) placed the monitor carefully on the patient's abdomen over the uterine fundus, an area believed to provide the most reliable information. Uterine activity was monitored for 1 hour before instillation of the PGE₂ and during seven subsequent 1-hour periods (0 to 1, 1 to 2, 2 to 3, 4 to 5, 6 to 7, 8 to 9, and 10 to 11 hours after dosing). Whereas all patients ambulated in the hospital after the first hour, recordings occurred as the patients reclined.

Monitoring data from each case were stored in the recording unit. The printout on the strip (paper speed, 3 cm/min) provided the following information: starting time and duration of monitoring, presence or absence of monitoring gaps, and warnings about any artifacts, poor connection, and end of transmission.

Four interpreters were used to assure reliability of interpretation. Uterine activity patterns as described by Sheerer et al. were characterized into two groups according to the amplitude of the contractions. Those that had an amplitude of <5 mm on the tocograph tracing and that occurred at 1- to 2-minute intervals represented a low-amplitude, high-frequency pattern. A second group of contractions consisted of those with a high-amplitude (≥6 mm), lasting at least 30 seconds and occurring less frequently. We also sought evidence for uterine hyperstimulation as described previously after PGE₂ treatment as a contraction frequency of >5 in 10 minutes or contractions exceeding a 2-minute duration. §

For each case, the proportion of time occupied by any contractions and the number of high-amplitude contractions were recorded for each hourly period. Nonparametric and parametric statistical evaluations included the χ^2 or Fisher exact test, Student t test, and analysis of variance. Results were expressed as mean \pm SD. A value of p < 0.05 was considered statistically significant.

Results

Forty women were enrolled between January and June 1990. None was excluded from data analysis. Twenty patients were assigned to the gel treatment group and 20 to the pessary group. No differences were found in demographic characteristics between the two groups (Table I).

Fig. 1 shows the mean hourly high-amplitude contraction frequencies and the proportion of time of any uterine activity (low-amplitude, high-frequency, or high-amplitude) for the two treatment groups. Both groups showed a statistically significant increase in uterine activity after dosing (Friedman analysis of variance,

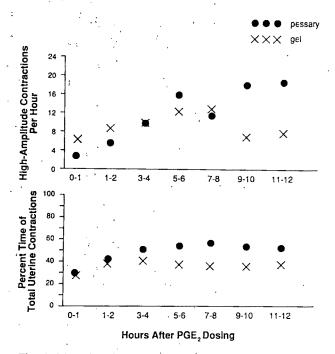


Fig. 1. Mean hourly high-amplitude contraction frequency and percent time of total uterine contractions for intravaginal PGE₂ pessary and gel-treated groups.

p < 0.001). All patients were identified as having increased uterine activity within the first hour. Both groups exhibited a crescendo of uterine activity in the first 6 hours after dosing.

For those treated with the gel, mean uterine activity reached a peak within the first 4 hours. Increases in high-amplitude uterine activity were observed in the first 1 to 2 hours after gel application and persisted during the II-hour observation period in 10 (50%) cases.

With the controlled-release pessary the peak in mean uterine activity was more delayed (at 5 to 8 hours after treatment) than with gel treatment. During the final observation period, high-amplitude contractions were observed every 3.2 ± 1.9 minutes on the average in the pessary group, compared with every 7.1 ± 2.6 minutes in the patients receiving gel (p < 0.05).

Table II illustrates the intrapartum outcomes of the two treatment groups. Labor as defined by uterine contractions leading to cervical change was initiated during the observation period in 18 (80%) of pessary cases and 10 (50%) patients receiving gel. Oxytocin was used for augmentation (in the presence of hypotonic contractions) or induction (absence of uterine contractions). Delivery within the 12-hour observation period occurred in six (30%) patients receiving the pessary and three (15%) receiving gel. Of the remaining 14 patients in the pessary group, six had sustained uterine activity by the end of the 12-hour observation period and did

Table I. Demographic characteristics of patients receiving low-dose PGE₂ intravaginally in the form of gel or sustained-release pessary

	Gel (n = 20)	$\begin{array}{c} Pessary\\ (n=20) \end{array}$
Maternal age yr)	25.5 ± 4.86	25.3 ± 4.95
Race (% white)	14 (70%)	16 (80%)
Parity (% nulliparous)	6 (30%)	11 (55%)
Gestational age (wk)	39.1 ± 1.74	40.3 ± 1.6
Reason for induction		
Postdates	4 (20%)	9 (45%)
Hypertension or preeclampsia	6 (30%)	3 (15%)
Fetal macrosomia or gestational diabetes	4 (20%)	4 (20%)
Other	6 (30%)	4 (20%)
Predose Bishop score	• •	•
0-2	12 (60%)	5 (25%)
3-4	8 (40%)	15 (75%)

None of the comparisons were statistically different (p > 0.05).

Table II. Intrapartum outcomes of patients receiving PGE2 in gel or pessary

	$Gel \\ (n = 20)$	$ \begin{array}{c} Pessary\\ (n = 20) \end{array} $
Bishop score at 12 hr after dosing	7.0 ± 3.7	9.7 ± 3.5
Labor ≤12 hr	10 (50%)	18 (80%)
Delivery ≤12 hr	3 (15%)	6 (30%)
Need for oxytocin		• •
None	9 (45%)	12 (60%)
Augmentation	2 (10%)	5 (25%)
Induction	9 (45%)	3 (15%)
Cesarean delivery	6 (30%)	4 (20%)

not require oxytocin, as did six of 17 patients in the gel group. These differences were not statistically significant.

Uterine hyperstimulation occurred in two (10%) of the pessary cases and none of the gel cases. Its onset began at 2.8 ± 1.3 hours after dosing in multiparous patients and at 3.7 ± 2.3 hours in nulliparous patients. No adverse maternal or fetal outcomes were apparent after removal of the pessary. All infants in both groups did well with no evidence of birth asphyxia (1-minute Apgar score <7; umbilical artery pH, <7.20). All 5-minute Apgar scores were ≥ 7 .

Comment

The success of PGE₂ preparations in preinduction cervical ripening has led to a considerable interest in understanding the mechanisms of its action. Microscopic and ultrastructural studies have indicated its principal effect to be a dissolution of collagen fibers and an increase in the ground substance within the cervix.¹⁰ Increases in uterine activity are also frequently seen with use of these medications. Granstrom et al.¹¹ demonstrated myometrial activity to increase significantly during the first 3 hours after intravaginal administration of a 4 mg PGE₂ gel through a flexible, thin microtransducer in the cervical canal.

This study represents what we believe to be the first quantitative assessment of uterine activity after two methods of PGE2 administration. Low-amplitude, highfrequency, and high-amplitude patterns of contractions were measured with new ambulatory, pressure-sensitive tocodynamometers. Significant increases in total uterine activity were found with both the PGE2 gel and pessary preparations. The early peak in total uterine activity with a single dose of the PGE2 gel supports our prior clinical impressions and that reported by Granstrom et al.11 Sustained uterine activity was less predictable, and labor began in half of these patients during the preinduction observation period. In contrast, those treated with the controlled-release PGE2 pessary showed a more gradual increase in total uterine activity that peaked later, approximately 5 to 8 hours after administration. These differences would suggest that the controlled-release preparation acted by initiating labor and promoting changes in cervical connective tissue.

Although differences in mean uterine activity between the two study groups were not statistically significant, labor began in more patients with the pessary than with the gel. In these cases 80% had high-amplitude contractions at the end of 11 hours and did not require the administration of oxytocin. Although the

incidence of uterine hyperstimulation was low, it may occur more frequently with the PGE₂ pessary group, unless the pessary is removed in a timely manner.

Observations from the current study provide more precise knowledge about patterns of uterine activity with low-dose intravaginal PGE2 preparations for preinduction cervical ripening. Uterine activity may be initiated by either method of delivery, with activity persisting more frequently in the controlled-release pessary group. These findings are important when one considers the duration of electronic fetal and uterine monitoring after dosing and the potential dismissal of patients from the hospital before the scheduled induction of labor.

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"Meconiumcrit" and birth asphyxia

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Determination of the percent by volume of the solid component of meconium (the "meconiumcrit") provides a more objective method of characterizing the type of meconium. In a study of 106 women with meconium-stained amniotic fluid, 61 (58%) had thin meconium, 36 (34%) had moderate meconium, and nine (8%) had thick meconium. There was no correlation between the type of meconium and newborn acidemia (umbilical artery pH, <7.20)—13%, 19%, and 11%, respectively. None of the newborns with either thin or thick meconium had 1-minute Apgar scores of ≤3 and only two with moderate meconium had such Apgar scores; none had an Apgar score of ≤3 at 5 minutes. None of the newborns with thin or moderate meconium had meconium aspiration syndrome, although two of nine infants with thick meconium did have meconium aspiration syndrome. All newborns subsequently did well and left the hospital in good condition. There would appear to be no correlation between the consistency of meconium and recently reported criteria for defining birth asphyxia. (AM J OBSTET GYNECOL 1991;165:1010-3.)

Key words: Meconiumcrit, birth asphyxia, meconium aspiration syndrome

Meconium is often used as a marker for intrapartum or birth asphyxia and emphasis has been placed on the consistency of meconium—thin, moderate, or thick. Although some investigators believe that meconium is not associated with fetal hypoxia, acidosis, or fetal distress and indeed may even be a normal physiologic event,1-5 others have postulated that meconium is a sign of fetal hypoxia and acidosis or "intrapartum asphyxia."6-11 The diagnosis of asphyxia often rests solely on the 1- and 5-minute Apgar scores, with mild to moderate asphyxia defined as a score of 4 to 6 and severe asphyxia as a score of 0 to 3.12 However, many different factors can affect the Apgar score, including gestational age, maternal medication, anesthesia used for delivery, and the personnel assigning the score (e.g., nurse, obstetrician, or pediatrician). Another important factor that can result in a low Apgar score, especially at 1 minute, is suctioning of the trachea, a common practice in the presence of meconium. This may be especially true in the presence of thick meconium where more vigorous suctioning may be required. It is important to note that the majority of newborns diagnosed as having asphyxia on the basis of Apgar scores have no detectable neurologic or intellectual sequelae.13-16

Another criterion used in defining birth asphyxia is the presence of newborn acidemia. Traditionally, new-

born acidemia has been defined as an umbilical artery pH of <7.20.18 This level may be arbitrarily high, however, because the lower pH range in normal uncomplicated pregnancies reportedly has been as low as 7.15 to 7.10.17, 18 Although the precise value required to define acidemia is not known, umbilical artery pH values of <7.0 probably more realistically represent clinically significant acidosis. 19, 20 Because asphyxia implies hypoxia leading to metabolic acidosis, the presence of acidemia at birth may provide a more objective method to evaluate the significance of meconium-stained amniotic fluid. The purpose of this study was to evaluate the association of the consistency of meconium as measured by the "meconiumcrit" and currently accepted markers of birth asphyxia, namely, the I- and 5-minute Apgar scores, umbilical artery cord blood pH, and newborn seizures.

Material and methods

Patients were included in this study if they were at term (birth weight, >2500 gm) and had meconiumstained amniotic fluid. There were 106 such women who had a 10 ml sample of amniotic fluid collected by an intrauterine pressure catheter after the first 10 ml was discarded. In addition to this sample, 57 women also had a second 10 ml sample of amniotic fluid collected by bedpan at the time of amniotomy to determine if the intrauterine catheter would diminish sample quality or quantity. All amniotic fluid specimens were placed in glass tubes and centrifuged at 1000 revolutions/min for 10 minutes. The meconiumcrit was measured by dividing the solid volume by the total volume, as with hematocrit, and the samples were graded as thin, moderate, and thick according to the solid component by volume (<10%, 10% to 30%, and 30%, re-

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Table I. Degree of meconium as graded by meconiumcrit compared with clinical estimate

Meconium	Clin estin		Mecon	iumcrit
	No.	%	No.	%
Thin	58	55	61	58
Moderate	38	36	36	34
Thick	10	9	9	8

Spearman's $\rho = 1.00$; Pearson's r = 0.997; p = 0.047.

spectively). The cutoffs for percent solid component for defining the three groups were arbitrarily selected before initiation of the study. Meconium also was graded subjectively as thin, moderate, or thick by the physicians in attendance but was unknown at the time of centrifugation and grading according to the meconiumcrit. Umbilical artery blood gas analysis was performed in all newborns. All samples were drawn immediately from a doubly clamped segment of umbilical cord at delivery, placed into ice, and transported directly to the laboratory for analysis. As is our delivery room policy, the obstetrician immediately suctioned the oropharynx with a bulb syringe after delivery of the fetal head. Pediatric house staff attend all deliveries complicated by meconium, and the vocal cords are visualized with a laryngoscope, followed by suctioning by a DeLee catheter. The designation of meconium below or above the vocal cords was based on pediatric assessment in the delivery room. The subsequent diagnosis of meconium aspiration syndrome was based on characteristic x-ray findings and the clinical course of the infant in the special care nursery. Apgar scores were assigned by the pediatric house staff in attendance. We stratified the umbilical pH results and Apgar scores into several groups. Statistical comparison was performed with the χ^2 test or Fisher's exact probability test where appropriate.

Results

With the use of the percent solid component by volume (i.e., the meconiumcrit), there were 61 (58%) patients with thin meconium, 36 (34%) with moderate meconium, and nine (8%) with thick meconium. The correlation of the degree of meconium as determined clinically compared with the meconiumcrit is summarized in Table I.

In addition, there was no difference in meconiumcrit on the basis of sample collection methods, bedpan versus intrauterine pressure catheter (Spearman's $\rho = 1.00$; Pearson's r = 0.998, p = 0.047). Three (5%) of the infants with thin meconium, six (14%) of the infants with moderate meconium, and two (22%) of the infants with thick meconium had 1-minute Apgar

Table II. Neonatal outcome and consistency of meconium in 106 pregnancies

	Г							
	Meconiumcrit							
Marker of	0%-10% (thin) $(n = 61)$		10%-30% (moderate) (n = 36)		>30% (thick) (n = 9)			
birth asphyxia	No.	%	No.	%	No.	%		
Apgar score								
1 min .<6	3	5	6	14	2	22		
≤3	0	0	2	6	0	0		
5 min	_	_	_	_				
. <6	1	2	1	3	1	11		
≤3	0	0	0	0	0	0		
Meconium aspiration	0	0	0	0	2	22		
Newborn seizures	0	0	0	0	0	0		

p, Not significant.

Table III. Mean umbilical artery pH and frequency of newborn acidemia

Maybon of	0%-10% (thin) (n = 61)		10%-30% (moderate) (n = 36)		>30% (thick) (n = 9)	
Marker of birth asphyxia	No.	%	No.	%	No.	%
Cord pH Mean ± SD	7.26 ±	- 0 04	7.95 +	: 0.05	7.24 ±	- 0.06
<7.20	8	13	7	19	1	11
< 7.00	0	0	0	0	0	0
<7.20 and 5 min Ap- gar score <6	0	0		0	0	0

p, Not significant.

scores <6 (Table II). Only two (6%) of the infants in the moderate meconium group had a 1-minute Apgar score ≤3. None of the infants with thin or thick meconium had Apgar scores ≤3 at 1 minute. One of the infants with thin meconium, one with moderate meconium, and one with thick meconium had 5-minute Apgar scores <6. None of the 106 infants had a 5minute Apgar score ≤3.

The average mean umbilical artery pH of the 106 infants was 7.25; this did not significantly vary among the three groups (Table III). Eight (13%) of the infants with thin meconium, seven (19%) with moderate meconium, and one (11%) with thick meconium had an umbilical artey pH of <7.20. No infant in the study had an umbilical artery pH of <7.00, a more accurate reflection of significant newborn acidemia. Also there were no infants with an umbilical artery pH <7.20 who also had a 5-minute Apgar score <6.

Statistical comparison of each of these parameters

including 1- and 5-minute Apgar scores, mean umbilical artery pH, frequency of newborn acidemia, occurrence of meconium aspiration, and newborn seizures did not reveal any significant differences among the three groups.

All the infants were born alive and there were no neonatal deaths. Meconium aspiration syndrome developed in two infants with thick meconium. The infants were treated with aggressive airway management and subsequently did well. None of the 106 infants suffered from newborn seizures.

Comment

Mitchell et al.,¹⁰ in a study of 53 women with moderate to thick meconium, reported that 53% of the newborns were acidotic at birth and the majority of them had metabolic acidemia. These authors further stated that meconium below the cords was associated with fetal metabolic acidosis and neurologic depression, implying intrapartum asphyxia. A relatively high umbilical artery pH cutoff of 7.25 was used to define acidemia in this study, and the criteria used to define metabolic acidemia were not given. Moreover, moderate to thick meconium was subjectively defined according to color of the amniotic fluid.

In an attempt to more objectively assess the meconium content of amniotic fluid, Weitzner et al.21 reported a technique for quantifying amniotic fluid, termed the meconiumcrit. These authors used a 3.0 gm specimen of meconium obtained from a newborn and diluted it with various quantities of amniotic fluid to obtain concentrations ranging from 1.5 to 10.0 gm/100 ml. They used standard hematocrit tubes for determining the meconiumcrit. While we agree that this technique is both simple and reproducible in a laboratory setting, we found it somewhat more difficult to use standard hematocrit tubes in the presence of meconium with particulate matter in a clinical setting. Thus we chose both a larger sample and larger tubes that were spun undiluted. Although our criteria for defining the three groups according to the meconiumcrit were arbitrarily selected, there was excellent correlation (r = 0.997) with the subjective assessment by the physician in attendance during labor and delivery.

In a previous report from our institution of 323 term pregnancies with meconium-stained amniotic fluid (incidence, 18%), Yeomans et al.²² reported that 21% of the newborns had an umbilical artery pH of <7.20, 7% had a pH <7.15, and none had a pH <7.00. There was no correlation between the type of meconium on the basis of the clinical estimate and pH results in this study. Using a more objective method of characterizing the type of meconium (i.e., the meconiumcrit), we likewise found no correlation between the character or type of meconium and the frequency of acidemia (defined

as either an umbilical artery pH <7.20 or <7.00), the type of acidemia (respiratory or metabolic), or low (\leq 3) 1- or 5-minute Apgar scores. Two of the nine newborns with thick meconium did have meconium aspiration syndrome; however, both were normal at discharge. Considering that only 2% of all newborns in most published series will have meconium aspiration syndrome, the determination of thick meconium by the meconiumcrit may prove a useful predictor of risks for this complication. However, the number of infants with thick meconium in this study is not large enough to address this issue in a statistical manner.

In conclusion, the percent solid component by volume of meconium as determined by the meconiumcrit would appear to provide an accurate method of assessment of the character or type of meconium. Using recently reported criteria to define birth asphyxia such as an umbilical artery pH of <7.00, an Apgar score of ≤3, and newborn neurologic dysfunction (i.e., seizures), there would also appear to be no correlation between the type of meconium and birth asphyxia.

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The relationship between umbilical artery Doppler velocimetry and fetal biometry

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The relationship between peak-systolic/end-diastolic ratio of the umbilical artery waveform and fetal biometry was studied in 127 uncomplicated pregnancies with established dates between 20 and 40 weeks' gestation. At each ultrasonographic examination fetal biometry included measurement of the biparietal diameter, head circumference, abdominal circumference, and femur length. The peak-systolic/end-diastolic ratio was measured by either a continuous or a pulsed-wave method. There were significant linear negative correlations between all the biometric parameters, as well as between the ultrasonographically estimated fetal weight and peak-systolic/end-diastolic ratio. Of the individual ultrasonographic parameters the femur length (for gestations <30 weeks) and the abdominal circumference (for gestations ≥30 weeks) were found to be best correlated with the peak-systolic/end-diastolic ratio. Regression curves, including the 10th and the 90th percentile, were developed between each biometric parameter (biparietal diameter, head circumference, abdominal circumference, and femur length), as well as between estimated fetal weight and peak-systolic/end-diastolic ratio. The estimated fetal weight nomogram had the best sensitivity (48%) in predicting intrauterine growth retardation. These nomograms should prove most useful in assessing downstream placental vascular resistance in high-risk patients with unknown dates. (AM J OBSTET GYNECOL 1991;165:1013-9.)

Key words: Umbilical artery velocimetry, fetal biometry

Continuous or pulsed-wave Doppler ultrasonography of the fetal umbilical artery has been increasingly used in clinical obstetrics in the management of highrisk pregnancy. ¹⁻³ The measurement of the peak-systolic/end-diastolic ratio of the umbilical artery flow velocity waveform is the most commonly used method. Abnormal ratios have been associated with an increased incidence of neonatal morbidity, mortality, and intrauterine growth retardation. ^{4, 5} The abnormal wave-

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forms are believed to be the result of increased downstream placental vascular resistance and therefore of impaired placental perfusion.⁶ The proper use of the umbilical artery peak-systolic/end-diastolic ratio, however, requires knowledge of the length of gestation, which is unknown or unavailable in 20% to 40% of pregnant patients.7 When the last menstrual period is unknown or uncertain, extrapolation of gestational age on the basis of fetal biometric data may lead to erroneous interpretation of the peak-systolic/end-diastolic ratio results, because there are no data regarding the relationship between the umbilical artery peak-systolic/end-diastolic ratio and fetal biometry. Therefore the purpose of the present report was: (1) to study the relationships and generate regression curves between the peak-systolic/end-diastolic ratio and the most commonly used fetal biometric parameters (biparietal di-

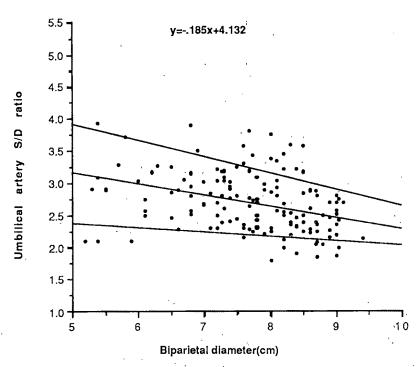


Fig. 1. Scattergram illustrating relationship (along with 10th, 50th, and 90th percentile lines) between umbilical artery peak-systolic/end-diastolic ratio (S/D) and biparietal diameter (R=0.395, p=0.0001).

ameter, head circumference, abdominal circumference, femur length, and estimated fetal weight); (2) to assess which fetal biometric parameters, if any, are significantly correlated with the peak-systolic/end-diastolic ratio; and (3) to determine the sensitivity of the fetal biometric parameter nomograms to predict intrauterine growth retardation (IUGR).

Material and methods

Patients were recruited from those referred to our antepartum fetal evaluation unit at the University of Connecticut Health Center. We used 127 uncomplicated pregnancies between 20 and 40 weeks of gestation (dates confirmed by early ultrasonography) for a single ultrasonographic assessment. The patients were referred for confirmation of clinical dates or fetal size. Patients were selected if they had initiated prenatal care early in pregnancy, if they had regular menstrual cycles with reliable menstrual dates, and if the results of ultrasonographic examination were consistent with their menstrual dates. All patients had intact membranes and singleton pregnancies. Patients were excluded if fetal anomalies were detected or if the estimated fetal weight was <-2 SD or >2 SD from the mean for gestational age.8

The ultrasonographic examinations were performed, using a model 128 (Acuson, Mountain View, Calif.) or a model 3000 or 2600 (General Electric, Rancho Cordova, Calif.). A 3.5 or 5 MHz linear-array trans-

ducer was used. All machines had freeze-frame capabilities and electronic calipers for measurement. The ultrasonographic velocity was 1540 m/sec. The study was cross-sectional, and each patient was included only once. Routine measurements (biparietal diameter, head circumference, abdominal circumference, and femur length) were obtained and recorded on each patient. The biparietal diameter and head circumference measurements were obtained from an axial scanning plane at the level of the thalami. The fetal abdominal circumference was taken perpendicular to the long axis of the fetal body at the level of the ductus venosus complex. The abdominal circumference was measured directly with electronic calipers or predominantly by using the formula $D1 + D2 \times 1.57$, where D is diameter (both methods have been shown to give equivalent results).9 The femur length was measured according to the technique described by O'Brien and Queenan.10 The ultrasonographic estimated fetal weight was calculated by averaging the estimated fetal weight obtained by both Shepard's (using biparietal diameter and abdominal circumference)11 and Hadlock's (using abdominal circumference and femur length)12 formulas. The umbilical artery peak-systolic/end-diastolic ratio was obtained transabdominally by continuous (model 5000A, Multigon, Mount Vernon, N.Y.) or pulsed-wave (model 128, Acuson) Doppler ultrasonography. All studies were performed during fetal apnea. The peak-systolic/end-diastolic ratios of three waveforms from three

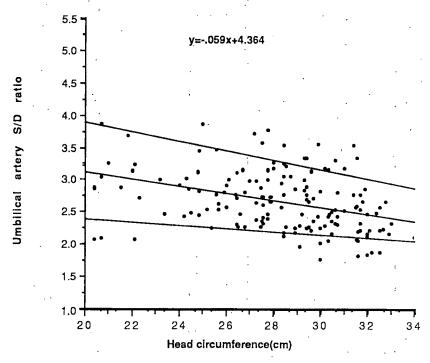


Fig. 2. Scattergram illustrating relationship (along with 10th, 50th, and 90th percentile lines) between umbilical artery peak-systolic/end-diastolic (S/D) ratio and head circumference (r = 0.422, p = 0.0001).

different sampling sites were computed, and the mean ratio was used in the analysis.

Scattergrams of the peak-systolic/end-diastolic ratio values versus gestational age and fetal biometry were developed. Regression analyses were used to establish nomograms, including the 10th, 50th, and 90th percentile lines. To assess which fetal biometric parameter(s), if any, best correlated with the peak-systolic/enddiastolic ratio, multiple regression analyses and stepwise regression analyses were used. A p value < 0.05. was considered statistically significant. The statistical analysis of the data was performed by computer (Macintosh II) with the Stat View 512+ (Abacus Concepts, Berkeley, Calif.) and CricketGraph (Cricket-Graph, Malvern, Pa.) statistical packages.

During the study period there were 25 patients with known dates (based on last menstrual period and confirmed by early ultrasonography) who had growthretarded fetuses (estimated fetal weights < -2 SD from the mean for gestational age).8 The gestational ages of these fetuses ranged from 26 to 38 weeks. The fetuses were used to determine the sensitivity of the newly constructed nomograms on the basis of fetal biometry, to predict growth retardation. In all 25 fetuses the diagnosis of IUGR was confirmed after birth.

Results

The study population consisted of 127 patients with gestational age (mean \pm SD) of 31 \pm 4 weeks (median,

31.1). Of these, 104 (81%) were white, 12 (10%) black, and 11 (9%) Hispanic. Seventy patients (55%) were nulliparous and 57 (45%) were multiparous. The maternal age (mean \pm SD) was 26.3 \pm 5.6 years (range, 15 to 40). The best-fit curves between peak-systolic/enddiastolic ratio and biparietal diameter, head circumference, abdominal circumference, femur length, and estimated fetal weight were linear. The r values were 0.395, 0.422, 0.461, 0.453, and 0.460, respectively (all p values were = 0.0001). The scattergrams, including the 10th, 50th, and 90th percentile boundaries, and the regression equations are illustrated in Figs. 1 to 5. The estimated fetal weight had the highest linear negative correlation with the peak-systolic/end-diastolic ratio (r = 0.461). The best-fit curve between the peaksystolic/end-diastolic ratio and gestational age also was linear (Fig. 6).

To determine the origins and to assess which fetal biometric parameters contribute to this negative correlation between estimated fetal weight and the peaksystolic/end-diastolic ratio, the data were analyzed according to two gestational age groups, <30 weeks' gestation (46 patients) and ≥30 weeks' gestation (81 patients). In both gestational age groups there was a significant linear negative correlation between estimated fetal weight and the peak-systolic/end-diastolic ratio (p < 0.05 and p < 0.0005, respectively). To assess which ultrasonographic parameter(s) significantly correlated with the peak-systolic/end-diastolic ratio within

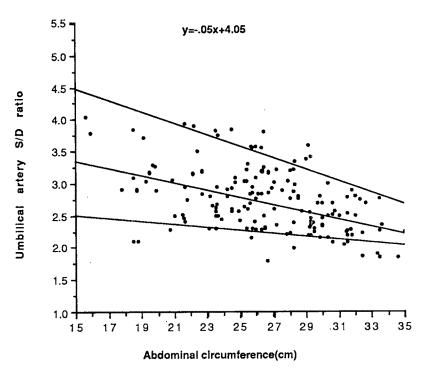


Fig. 3. Scattergram illustrating relationship (along with 10th, 50th, and 90th percentile lines) between umbilical artery peak-systolic/end-diastolic (S/D) ratio and abdominal circumference (r = 0.460. p = 0.0001).

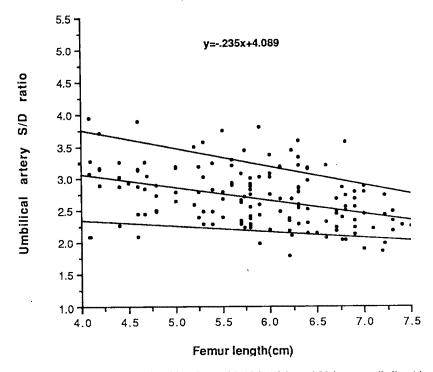


Fig. 4. Scattergram illustrating relationship (along with 10th, 50th, and 90th percentile lines) between umbilical artery peak-systolic/end-diastolic (S/D) ratio and femur length (r = 0.453, p = 0.0001).

each gestational age group. multiple regression analyses and stepwise regression analyses were used with the peak-systolic/end-diastolic ratio used as the dependent (outcome) variable and biparietal diameter, head

circumference, abdominal circumference, and femur length used as the independent (predictors) variables.

Multiple regression analysis of the group <30 weeks' gestation revealed that the overall association was sig-

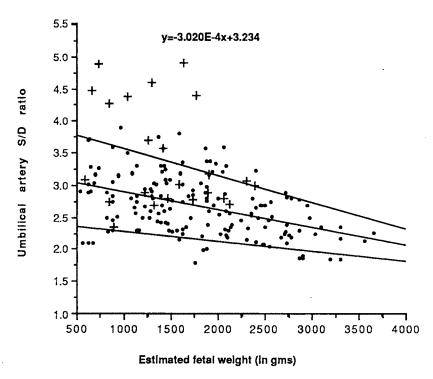


Fig. 5. Scattergram illustrating relationship (along with 10th, 50th, and 90th percentile lines) between umbilical artery peak-systolic/end-diastolic (S/D) ratio and estimated fetal weight (r = 0.461,p = 0.0001). Plus sign, Growth-retarded fetuses.

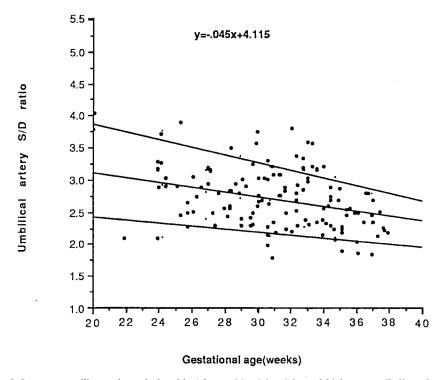


Fig. 6. Scattergram illustrating relationship (along with 10th, 50th, and 90th percentile lines) between umbilical artery peak-systolic/end-diastolic ratio and gestational age (r = 0.391, p = 0.0001).

nificant (p < 0.05). Of the four independent variables, only femur length was significantly correlated with the peak-systolic/end-diastolic ratio (p < 0.05; β coefficient, -0.477; standard error, 0.211; standard coeffi-

cient, -0.673; t value, 2.262). Stepwise regression analysis of this group of fetuses of <30 weeks' gestation also indicated that the femur length was the only independent variable to enter the equation (r = 0.477, $R^2 = 0.227$, adjusted R^2 ; = 0.21). Similar analyses were performed for the group of fetuses with gestational ages ≥30 weeks. With multiple regression analysis it . was found that the overall correlation of the independent variables to the peak-systolic/end-diastolic ratio was significant (p < 0.0005). Of the individual independent variables only abdominal circumference was significantly associated with the peak-systolic/end-diastolic ratio (p < 0.005; β coefficient, -0.086; standard error, 0.03; standard coefficient, -0.534; t value, 2.896). Stepwise regression analysis also was performed, and only the abdominal circumference entered equation $(r = 0.525, R^2 = 0.276, \text{ adjusted})$ $R^2 = 0.267$). The same analyses were repeated with gestational age included as one of the independent variables, but the results were the same. Even when gestational age was used as one of the independent variables, only femur length for fetuses <30 weeks' gestation and only abdominal circumference for fetuses ≥30 weeks' gestation again were the only variables significantly correlated with the peak-systolic/enddiastolic ratio.

With the established nomograms between the peaksystolic/end-diastolic ratio and the fetal biometric parameters, the sensitivity of each parameter to detect IUGR was calculated. The gestational age of the fetuses with IUGR was $32.7 (\pm 3.6)$ weeks (median, 33). Two of the fetuses had absent end-diastolic velocities and were excluded from calculations of sensitivity. The sensitivity of detecting growth retardation by using the biparietal diameter nomogram was 35% (8/23), head circumference 35% (8/23), abdominal circumference 35% (8/23), femur length 30% (7/23), and estimated fetal weight 48% (11/23). Of these ultrasonographic parameters, the estimated fetal weight versus the peaksystolic/end-diastolic ratio had the best sensitivity. The sensitivity by using the gestational age versus the peaksystolic/end-diastolic ratio nomogram was 52% (12/23).

Comment

In 1978 McCallum et al.¹⁸ suggested that umbilical artery velocity waveforms could be recorded by ultrasonographic Doppler probes. Subsequently various investigators suggested that the calculation of peak-systolic/end-diastolic ratio of the umbilical artery velocity waveform reflects placental vascular resistance.⁶ Elevated ratios have been associated with an increased incidence of adverse perinatal outcome, such as growth-retarded infants who are prone to hypoxic complications, twin-to-twin transfusion, and preeclampsia.^{4, 5, 14, 15} It has been clearly shown from previous investigations that there is a steady decline in the peak-systolic/end-diastolic ratio with advancing gestational age, ^{16, 17} the length of gestation should be known for the accurate interpretation of the ratio result. How-

ever, the last menstrual period is unknown or uncertain in approximately 20% to 40% of pregnant patients.⁷

This is the first report to evaluate the relationships and establish nomograms between each of the most commonly used ultrasonographic parameters (biparietal diameter, head circumference, abdominal circumference, femur length) and the estimated fetal weight and peak-systolic/end-diastolic ratio. Statistically significant relationships were found between the ratio and each of the ultrasonographic parameters as well as the estimated fetal weight. There was an inverse relationship between the ratio and each ultrasonographic parameter. The use of these gestational age-independent nomograms may prove useful for evaluating placental resistance in patients with unknown dates or late registrants with uncertain dates. In the 23 fetuses with IUGR encountered during the study period, the sensitivity of the fetal biometry versus the peak-systolic/end-diastolic ratio nomograms to detect IUGR was not significantly different from that with the gestational age versus the peak-systolic/end-diastolic ratio nomogram. The best sensitivity was achieved by the estimated fetal weight versus the ratio nomogram (48%), which compared quite favorably with the sensitivity obtained by using gestational age (52%). The detection of an abnormally high peak-systolic/end-diastolic ratio in growth-retarded fetuses depends on whether small fetal size is the result of impaired uteroplacental blood flow or nonplacental causes. Furthermore, even with uteroplacental insufficiency the ratio still may be normal in some cases. Therefore it is not surprising that the sensitivity of detecting IUGR by our method is approximately 50% because many of the fetuses that weigh <10th percentile for gestational age may suffer lesser-degrees of uteroplacental insufficiency or the low weight may be the result of nonplacental causes.

Of interest were the results of the multiple and stepwise regression analyses that used the peak-systolic/end-diastolic ratio as the dependent (outcome) variable and the remaining ultrasonographic biometric parameters as the independent variables. One of the most interesting results of this study was the only significant correlation of femur length to the peak-systolic/enddiastolic ratio for fetuses <30 weeks and of abdominal circumference to peak-systolic/end-diastolic ratio for fetuses ≥30 weeks. These findings persisted even when the gestational age was included as one of the independent variables. The explanation for these findings lies in the fact that femur length is an indirect measure of fetal length and abdominal circumference is an indirect measure of fetal body weight. It is already known that the fetus exhibits two growth spurts, one in fetal length during the second trimester and the other in body weight during the third trimester. 18, 19 A breakpoint of 30 weeks was chosen in the analysis of the data because, as has been previously shown,18,19 the fetal

growth spurt in length has been almost completed while the growth spurt in weight has just begun by 30 weeks of gestation. Therefore it is not surprising to find a significant correlation between femur length, which represents fetal length, and the peak-systolic/end-diastolic ratio at gestational ages <30 weeks and between abdominal circumference, which represents fetal body weight, and the peak-systolic/end-diastolic ratio for gestational ages ≥30 weeks. It can be speculated that the lower the ratio, the lower the vascular placental resistance and thus the higher the uteroplacental blood flow, resulting in increased fetal growth, which is reflected by increased fetal length (or femur length) during the second trimester and increased body weight (increased abdominal circumference) during the third trimester. The finding of best correlation between the peak-systolic/end-diastolic and the femur length in gestations <30 weeks (and between the peak-systolic/enddiastolic ratio versus abdominal circumference in gestations ≥30 weeks) should not be interpreted that the femur nomograms should be used at <30 weeks and the abdominal circumference nomograms at ≥30 weeks. Our data simply suggest that the estimated fetal weight nomogram is similar to the gestational age nomogram, which is used widely, and that the greatest sensitivity for IUGR is achieved when the estimated fetal weight nomogram is used. As far as the clinical relevance of the other nomograms, (biparietal diameter, head circumference, abdominal circumference, and femur length vs the peak-systolic/end-diastolic ratio), these may be used in patients with fetal congenital anomalies that preclude the use of the estimated fetal weight nomogram (i.e., hydrocephalus, skeletal dysplasias, hydrops, etc.). In such cases a judgment should be made as to which biometric parameter is not affected by the fetal malformation, and the corresponding nomogram should be used.

In summary, this study supports the use of gestational age-independent nomograms with fetal biometry versus the peak-systolic/end-diastolic ratio used to detect fetuses with increased placental vascular resistance. The use of these nomograms should prove useful in evaluating patients with unknown dates. It also sheds some light into the relationship between fetal biometric parameters and placental vascular resistance. Placental vascular resistance as determined by the peak-systolic/end-diastolic ratio is more closely correlated with fetal size parameters than with gestational age. The fact that appropriately grown fetuses of the same gestational age may have different peak-systolic/end-diastolic ratios may be due to differences in placental vascular resistance and therefore nutrition. To investigate this possibility the next logical step should be an assessment of the correlation between fetal biometry and the peak-systolic/end-diastolic ratio in fetuses of the same gestational age. The value of the fetal biometric nomograms to assess IUGR is currently being investigated at our institution in a prospective manner.

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Risk factors for cordocentesis and fetal intravascular transfusion

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There is little information on the impact of technical aspects or patient characteristics on the risks of accessing the fetal circulation. We performed 594 diagnostic cordocenteses and 156 intravascular transfusions over 6 years. Pancuronium was administered during 52% of procedures. The number of needle punctures per successful procedure was unrelated to the placental location. However, the number of punctures required was lower if the placental cord origin rather than a midsegment was targeted (p < 0.0001). Bleeding from either the uterine or umbilical cord puncture site was not believed to be clinically significant, although the duration of bleeding was greater after arterial puncture than after venous puncture (p = 0.01) and after intravascular transfusion than after diagnostic cordocentesis (p < 0.0001). Amnionitis (suspected plus verified) complicated 0.5% of procedures. Preterm premature rupture of membranes (with or without amnionitis) followed 0.4% of procedures. Fetal bradycardia occurred in 6.6% $(6.6 \pm 0.8 \text{ minutes})$; range, 0.1 to 35 minutes). There were five perinatal losses after a diagnostic procedure, yielding an uncorrected loss rate of 0.8% (5/594). Each was associated with a prolonged bradycardia; each fetus was ultimately demonstrated to have been unsalvageable. Two independent risk factors for bradycardia were identified-arterial puncture and severe, early onset intrauterine growth retardation. The administration of pancuronium reduced the incidence of bradycardia in appropriately grown fetuses (6% to 1.5%; ρ < 0.05), but did not alter the incidence in growth-retarded fetuses. We conclude that cordocentesis performed with a needle guide is a safe procedure but that its risk varies with both the indication and the vessel punctured. (AM J OBSTET GYNECOL 1991;165:1020-5.)

Key words: Pregnancy, cordocentesis, fetal intravascular transfusion, complications

Since first reported in 1983,1 ultrasonographically guided percutaneous access of the fetal umbilical circulation has become common. The potential applications of the procedure, variously called cordocentesis, percutaneous umbilical blood sampling, or funipuncture, are many.2 However, as with any obstetric procedure, these applications will be limited by the associated perinatal morbidity and mortality. Several investigators have reported global perinatal loss rates for cordocentesis,⁸⁻⁷ but there is little information as to whether technical aspects of the procedure or patient characteristics have an impact on the risk. A prior report from this unit after our first 100 diagnostic cordocenteses noted that the risk of fetal bradycardia was higher after arterial puncture than after venous puncture.3 The purpose of this investigation was to determine, from a 6-year experience encompassing 750 diagnostic and therapeutic procedures, what factors were associated with an adverse outcome.

Material and methods

The fetal circulation was accessed 750 times (594 diagnostic cordocenteses in 375 pregnancies, 156 intravascular transfusions in 54 pregnancies) for a wide variety of indications.3 The techniques for cordocentesis and intravascular transfusion have been previously detailed.^{8, 8} Each used a 22-gauge spinal needle held in the ultrasonographic plane of a 5 MHz sector transducer by a fixed needle guide (Civco, Kalona, Ia.). With this apparatus, needle movement was limited to the vertical plane. The placental origin of the umbilical cord was the preferred target, unless the indication was red blood cell alloimmunization and the placenta was anterior. In that instance, or when the placental umbilical cord origin was not readily identified or if access was blocked by either a fetal part or the umbilical artery, a free-floating loop of cord away from the placenta with the umbilical vein in an anterior position was targeted. The fetal cord origin was rarely selected because of its proximity to the body and because it is innervated. Pancuronium (0.3 mg/kg estimated fetal weight) was administered intravenously whenever fetal movement

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was believed to pose a risk. The pressure of both the vessel punctured and the amniotic cavity were routinely measured, as previously described, through the same puncture.9, 10 The vessel punctured was definitively identified by a combination of the ultrasonographic image, the vessel blood pressure, and the direction traveled by an injected saline solution bolus. With the described methods diagnostic cordocentesis (including blood and amniotic cavity pressure measurement) was completed within 90 seconds of uterine puncture and intravascular transfusion was accomplished within 45 minutes. Prophylactic antibiotic therapy was not given. Transfusions were undertaken when the fetal hematocrit declined below 30% and consisted of red blood cells suspended in normal saline solution to yield a hematocrit of 70%.8 Five physicians performed the procedures described, although 83% were performed by one individual (C.W.).

All procedures were preceded by a detailed ultrasonographic examination that included interrogation of the umbilical artery with pulsed gated Doppler (General Electric 3600, Rancho Mirage, Calif.). At least three waveforms between fetal respirations were measured and the result was averaged. By convention, a systolic/diastolic ratio of ≥10 was assigned a value of 10.11

A complete blood cell count was performed on blood anticoagulated with ethylenediametetraacetate (Technicon H-1). The umbilical pH, PcO2, and PO2 were measured in heparinized blood (Instrumentation Laboratory) within minutes of the sample aspirations.

Severe, early onset intrauterine growth retardation was defined as either that ultrasonographically detectable by 32 weeks' gestation or symmetric growth retardation detected at any length of gestational age.12 In our unit this requires both an abdominal circumference percentile below 2.5 and an estimated weight percentile below 10.

A record of each procedure was maintained prospectively in a computerized data base whose accuracy was verified on a regular basis. This record included, in addition to the laboratory parameters, the indication(s) for the procedure, gestational age, placental location, the operator, number of successful and failed punctures, the location of the punctures, the vessel punctured, the duration of fetal bradycardia (heart rate <100 beats/min), the duration and site of bleeding, rupture of membranes within 2 weeks of the procedure, the need for emergency delivery, and perinatal loss. Results are presented as the mean \pm 1 SD unless otherwise stated. Statistical analyses included paired and unpaired t tests, Fisher's exact test, and stepwise multiple regression. A $p \le 0.05$ was considered to indicate either a significant difference between two means or the presence of a significant relationship between two variables.

Results

The most common indications per diagnostic procedure and per patient are shown in Table I. Indications for fetal transfusion included alloimmunization (n = 141), human parvovirus infection (n = 2), twintwin transfusion (n = 10), and fetal-to-maternal hemorrhage (n = 3). The mean \pm SD gestational age at the time of the procedure was 28.4 ± 5 weeks (range, 16 to 42 weeks). Sixty percent (n = 450) of procedures were performed at the placental cord origin, 38% (n = 283) in a free-floating loop of cord, and 2% (n = 17) at the fetal cord origin. There were no differences in the distribution of puncture sites between diagnostic and therapeutic procedures. Pancuronium was administered during 52% of the procedures. All transfused fetuses received pancuronium.

The mean number of skin insertions per successful procedure was 1.5 ± 0.8 . A third of aborted skin punctures were stopped before the needle entered the amniotic cavity. The number of punctures was unrelated to placental location and operator experience but was significantly lower when the placental cord origin was targeted, compared with all other locations (1.3 \pm 0.8 vs 1.7 ± 1 ; p < 0.0001).

Bleeding occurred from both uterine (12%) and umbilical cord (29%) puncture sites. Twenty fetuses had a platelet count <50,000/µl³; eight had a count <10,000/µl3. There was no correlation between the fetal platelet count and either the incidence or the duration of bleeding from the puncture site, even when the analysis was confined to fetuses whose platelet counts were <100,000/µl³. When bleeding did occur, the mean duration was greater after transfusion $(0.36 \pm 0.9 \text{ minutes})$ than after a diagnostic cordocentesis (0.13 \pm 0.3 minutes; p < 0.0001). Furthermore, the duration of bleeding from the puncture site was longer after arterial puncture than after puncture of the umbilical vein for both diagnostic and therapeutic procedures (each p = 0.01). The duration of bleeding from the uterine wall did not vary by the vessel punctured, but it was longer after transfusion than after cordocentesis (0.24 \pm 0.9 vs 0.10 \pm 0.4 minutes; p = 0.004). There was no direct or indirect evidence that any of the blood loss was clinically significant.

Culture-proved amnionitis (N = 3) and suspected amnionitis (N = 1) occurred after diagnostic (N = 2)and the rapeutic (N = 2) procedures. Three of the four followed a procedure that had required a single puncture and one followed a two-puncture procedure. In all instances the organism cultured was Staphylococcus epidermidis. All patients were seen in preterm labor. The presentation was characterized by malaise, myalgia, and a low-grade pyrexia beginning 5 to 10 days after the procedure. In all instances the women initially assumed they had acquired a viral illness. Although parenteral 1022 Weiner et al.

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Table I. Major indications	for diagnostic core	docentesis ($n=594$)	procedures among 375	patients)

	Per procedure		Per patient†	
Indication	%*	No.*	%*	No.*
Red blood cell alloimmunization	48	283	36	134
Rapid karyotype	38	227	61	227
Infection	25	146	36	136
Severe, early onset intrauterine growth retardation	16	96	24	90
Fetal curarization	9	27	9	17

^{*}Categories not mutually exclusive.

Table II. Risk factors for fetal bradycardia

Vessel punctured	Incidence (%)	
Diagnostic cordocentesis		
AĬl	6.1	
Umbilical vein		3.5
Umbilical artery		19.0*
Appropriately grown	4.1	
Úmbilical vein		1.7
Umbilical artery		15.7*
Severe growth retardation	17.2	
Umbilical vein		13.2†‡
Umbilical artery		35.3*§
Intravascular transfusion		
All	7.8	
Umbilical vein		4.8
Umbilical artery		$29.4\ $

^{*}p < 0.0001, compared with umbilical vein.

antibiotic therapy coupled with tocolysis was attempted in three instances, all infants were delivered prematurely. There were no deaths.

Preterm premature rupture of membranes within 7 days of the procedure occurred only after intravascular transfusion (N=3). Each patient was ≥ 34 weeks' gestation. There was no evidence of a learning curve for any of the practitioners in terms of the above complications.

The incidence of a clinically significant fetal brady-cardia (rate, <100 beats/min) was 6.6% (mean duration \pm SD, 6.6 \pm 8 minutes; range, 0.1 to 35 minutes). Thus the principal complication of accessing the fetal circulation for either diagnostic or therapeutic purposes was fetal bradycardia. All perinatal losses (and emergency deliveries) after a diagnostic procedure were directly the result of a prolonged procedure-related bradycardia (>15 minutes). In total, nine fetuses required emergency delivery, two with transfusion of either red blood cells or platelets when the umbilical artery was punctured, one because of hemolytic disease, and six because of early onset intrauterine growth retardation. Three of these fetuses had a chro-

mosome abnormality. The mean length of gestation at cesarean delivery of the karyotypically normal fetuses was 33 ± 3 weeks. None of these neonates were lost. Of the fetuses that experienced a bradycardiac episode that resolved without delivery, there were no apparent sequelae.

Two risk factors for bradycardia were identified (Table II). First, the incidence of bradycardia differed by the vessel punctured (umbilical artery, 21%; umbilical vein, 3.4%; p < 0.0001). There was no significant difference between diagnostic and therapeutic procedures in either the incidence of bradycardia or the resulting increase in the risk of bradycardia associated with arterial puncture. Fetuses who experienced bradycardia were similar in gestational age to those who did not.

Second, the incidence of bradycardia differed between appropriately grown and severely growthretarded fetuses, regardless of whether the artery or vein was punctured (Table II). No other diagnostic category (as defined in Table I) had an impact on the risk of bradycardia. In spite of similar ages, fetuses who experienced a procedure-related bradycardia had lower umbilical venous pH (7.38 \pm 0.05 vs 7.41 \pm 0.03; p < 0.0001), lower umbilical venous Po₂ $(32.6 \pm 16 \text{ vs } 38.3 \pm 8; p = 0.002)$, and higher umbilical venous Pco_2 (39.5 ± 6 vs 35.5 ± 4; p < 0.0001) than fetuses who did not experience a bradycardia. The umbilical artery systolic/diastolic ratio before the procedure was higher in fetuses in whom bradycardia developed (5.3 \pm 5 vs 3.9 \pm 3; p = 0.01). Of fetuses with absent umbilical artery diastolic flow, 21.4% had a procedure-related bradycardia, compared with 5.3% of those with any diastolic flow (p = 0.05). The incidence of arterial puncture was similar in both growthretarded and appropriately grown groups. Both growth-retarded and appropriately grown fetuses had a higher incidence of bradycardia when the umbilical artery was punctured.

Bradycardia was significantly more common after puncture of a midsegment than after puncture of the placental cord origin (10.8% vs 4%; p = 0.02). However, the likelihood of an arterial puncture also was significantly increased (19.8% vs 13.1%; p = 0.01). Fur-

[†]First procedure only.

 $[\]dagger p = 0.03$, compared with umbilical vein.

 $[\]ddagger p < 0.0001$, compared with appropriately grown fetuses.

p < 0.05, compared with appropriately grown fetuses.

^{||}p| < 0.002, compared with umbilical vein.

ther, growth retardation was disproportionately represented in the midsegment puncture group, no doubt because of the associated oligohydramnios. Therefore puncture of the midsegment itself did not appear a risk factor for bradycardia.

· On several occasions the umbilical artery was interrogated with pulsed Doppler during a bradycardiac episode. The vessel walls appeared thickened on realtime examination and either the systolic/diastolic ratio was increased over the preprocedure value or the diastolic flow was absent. As the heart rate began to normalize, diastolic flow returned.

There appeared to be a decrease in the incidence of fetal bradycardia when pancuronium was given (3.8% with pancuronium vs 7.9% without; p = 0.06, not significant). Unfortunately, the seemingly protective effect of pancuronium was confined to the group already at lowest risk for bradycadia—the appropriately grown fetus (6.0% without vs 1.5% with pancuronium, p < 0.03). There was no change in the incidence of bradycardia in severely growth-retarded fetuses after pancuronium (18.2% without vs 15.4% with pancuronium; p = 0.2, not significant). In addition, the effect of pancuronium did not vary with the vessel punctured. Almost all fetuses transfused received pancuronium, so that a similar evaluation of pancuronium for the therapeutic procedure group could not be done.

There were five procedure-related perinatal losses after a diagnostic procedure, yielding an uncorrected loss rate of 0.8% (5/594). Each of the five perinatal losses occurred in growth-retarded, hypoxemic, acidemic fetuses. Three of the five fetuses had a lethal trisomy, one had renal agenesis, and the fifth was severely growth retarded because of uteroplacental dysfunction with an estimated weight <500 gm at 29 weeks (460 gm at birth). In the latter instance the patient declined emergency cesarean delivery. Since none of these fetuses were likely to be salvageable, the corrected loss rate for diagnostic cordocentesis was 0%. The overall loss rate associated with intravascular transfusion was 3.6%. We have recently reported the results of fetal intravascular transfusion for the treatment of hemolytic disease in detail and will not duplicate it here.18 No fetus transfused because of a Coombs-negative anemia was lost. One additional appropriately grown fetus died 2.5 weeks after a diagnostic procedure and 3 days after an ultrasonographic examination had documented continued normal fetal growth. No evidence of either trauma, infection, or placental abnormality was observed at delivery shortly thereafter. The cause of the loss remains unknown, but we have not attributed it to the procedure. There were no other perinatal losses. Follow-up is 100%.

Comment

Whereas ultrasonographically guided percutaneous access to the fetal umbilical circulation has become common, factors contributing to losses and complications of the procedure remain largely unexplored. Our experience demonstrates that the risk of cordocentesis is highly influenced by the indication and the vessel punctured.

The major significant complication of cordocentesis was fetal bradycardia, leading either to perinatal death or to preterm delivery. Two independent risk factors for cordocentesis were identified, umbilical artery puncture and severe, early onset growth retardation.

The exact stimulus for the bradycardia is unknown. We have observed on several occasions a divergence in the systolic/diastolic ratio of the two umbilical arteries (including loss of diastolic flow in the punctured vessel) during the bradycardiac episode, which resolved as the heart rate returned to normal. This observation, coupled with the increased risk of bradycardia with arterial puncture, the increased echogenicity of the vessel during the episode, and the lack of an effect of uterine contractions on the umbilical artery systolic/diastolic ratio, suggests that the stimulus for the procedurerelated fetal bradycardia is localized arterial spasm at the site of puncture.

Severe, early onset growth retardation was an indepenent risk factor for bradycardia, because umbilical artery puncture occurred with equal frequency in growth-retarded and non-growth-retarded groups. There may well be a physiologic explanation for this observation. Hypoxia was most commonly observed in growth-retarded fetuses, and there was a relationship between bradycardia and suboptimal umbilical blood gas values. Arteries from various organs studied under hypoxic conditions in vitro have a tendency toward spontaneous contraction and an exaggerated response to catecholamines. We suggest that in vivo the umbilical arteries of hypoxic fetuses were similarly "twitchy." The observation that the systolic/diastolic ratio before the procedure was higher in fetuses who experienced bradycardia was consistent with our prior documentation of a relationship between the umbilical venous Po2 and an elevated systolic/diastolic ratio in potentially viable fetuses.11

Bleeding from the cord puncture site was not a clinically significant problem after any procedure in this series. However, significant fetal blood loss has been observed after cordocentesis.14, 15 Therefore it would be important to avoid factors associated with increased fetal blood loss whenever possible. Whether the lack of clinically significant bleeding in this series reflects a low number of indications associated with fetal hemorrhage (such as hemophilia and alloimmune thrombocytopenia), the method (freehand vs a needle guide), the needle size (22 gauge in our series vs 18 or 20 gauge), or the vessel punctured cannot be stated with certainty. However, eight fetuses with profound thrombocytopenia (<10,000 μl^s) were sampled without incident, and

the incidence and duration of bleeding from the puncture site of fetuses with a platelet count $<100,000/\mu l^3$ were unrelated to platelet number. We have not as yet tested a fetus with hemophilia or Glanzmann's thrombasthenia. Technique is a potential explanation for the absence of either a hemorrhagic or a thrombotic complication in this series. The percent of procedures complicated by a significant rise in the maternal serum α -fetoprotein concentration was greater after freehand intravascular transfusion.¹⁶ than after needle-guided intravascular transfusion.¹⁷ This suggests that lateral mobility permitted by a freehand technique increases the likelihood of trauma.

Since technique may have an impact on the complication rate, it is also appropriate to ask whether it could alter the overall loss rates. There are only two other large published series. Daffos et al. 6 (N=606) reported a 1.9% procedure-related loss rate and Boulot et al. 5 (N=322) a 3.1% loss rate. Each of these centers used a freehand technique. In spite of the fact that severe growth retardation was an uncommon indication for cordocentesis in either series, their combined loss rate was significantly greater ($\chi^2=4.0$, p=0.04) than the 0.8% uncorrected loss rate of this series.

Pancuronium seemed to protect against bradycardia by a mechanism that did not depend on the vessel punctured. There are several potential explanations that are not mutually exclusive. First, pancuronium increased the fetal heart rate. The fetal cardiac output has been reported to be essentially rate dependent. It may be that the vessel spasm induced by puncture was modest enough to be overcome by the increased blood flow. Second, intravenous pancuronium resulted in almost immediate paralysis of the fetus. Even small movement of the umbilical cord while the needle is in place could traumatize the smooth muscle and cause spasm. The latter explanation is supported by the observation that the protective effect of pancuronium did not vary with the vessel punctured.

We observed that the duration of bleeding from the umbilical cord varied with the vessel sampled. Arterial punctures bled longer, likely because of higher blood pressure. While the precise quantity of blood lost from the puncture wound cannot be estimated, it was not associated with a declining hematocrit after serial procedures. The increased duration of bleeding from the umbilical cord puncture site after transfusion may reflect either the development of a larger hole in association with the inevitable movement of the needle that must occur during any long procedure or dilution of platelet and soluble clotting components, as has been previously documented.¹⁹

Reports from the North American percutaneous umbilical blood sampling registry suggested that the longer a procedure took to complete, the greater the risk of

a complication, usually amnionitis.²⁰ The contributors to this registry all used a freehand technique. Time was not a relevant variable in this series. Either the vessel was punctured immediately or it was missed and the needle was removed. The incidence of amnionitis here was lower than that reported by other series.^{5, 6}

This series confirms that the umbilical vein is the target of choice for cordocentesis. Although in theory the umbilical artery might be preferred to the vein for the evaluation of growth retardation, the increased risk of bradycardia engendered by puncturing the artery outweighs, in our opinion, any potential benefit. The overall 83% success rate achieved for umbilical vein puncture demonstrates that the vessel punctured usually can be chosen and is not a chance event. However, in our experience vein puncture requires a willingness to target a midsegment when the umbilical arteries lie on top of the vein at the placental cord origin.

Because the risk of bradycardia varies with the indication, the consent form should be modified to reflect the indication. The risk of cordocentesis when the fetus is appropriately grown appears very low. When the fetus is severely growth retarded, the fetal weight should be estimated and the patient asked *before* the procedure to decide what should be done if a profound bradycardia results. We presently do not recommend cesarean section if the fetus is previable or has an estimated weight <500 gm.

There are instances, such as red blood cell alloimmunization or a fetal anomaly, where information obtainable with cordocentesis can be substituted by that obtainable with amniocentesis. In those circumstances it is appropriate to question whether the risks of cordocentesis outweigh its benefits. Clearly cordocentesis is more dangerous than amniocentesis. Bradycardia is not a major risk factor for amniocentesis. However, the risk and effects of bradycardia are probably the main difference between the two procedures. When a needle guide is used, the only difference between amniocentesis and cordocentesis is the target. Movement of the needle is essentially confined to the vertical plane. The other risks of needle puncture are the same regardless of the target. There are no modern series of latesecond-trimester to mid-third-trimester amniocentesis; however, our institution performs genetic amniocentesis with the same methods. The incidence of chorioamnionitis and rupture of membranes is virtually identical to that observed in this series. It is illogical to assume that the risks of premature rupture of membranes or chorioamnionitis are significantly less in the third trimester. We believe that if bradycardia is preventable, the risk of cordocentesis performed with a needle guide is the same as that with amniocentesis.

In summary, cordocentesis is a safe procedure. However, the risk does vary with both the vessel punctured and the state of fetal health. The loss rate experienced by a center will vary with the population composition. Further investigation of both the mechanism underlying fetal bradycardia and methods to prevent it are urgently needed.

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Fetal blood sampling in patients undergoing elective cesarean section: A correlation with cord blood gas values obtained at delivery

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The purpose of this study was to compare blood gas parameters obtained after delivery and cord clamping with those obtained in the intact fetal circulation. Eighteen patients undergoing elective cesarean section at term were studied. Before the induction of epidural anesthesia, cordocentesis was performed under ultrasonographic guidance. Subsequently, a second sample was obtained for cord blood gas analysis after delivery. Umbilical venous blood was analyzed for pH, Po₂, Pco₂, and base excess. When comparing samples obtained from the intact fetal circulation with those obtained after delivery, there was a significant difference in pH (7.36 \pm 0.03 vs 7.31 \pm 0.04; p < 0.0001), Pco₂ (41.15 \pm 3.66 vs 46.29 \pm 5.71; p < 0.018), Po₂ (32.92 \pm 8.54 vs 26.97 \pm 4.43, p < 0.02), and base excess (-0.79 \pm 1.19 vs -2.36 \pm 1.48; p < 0.0003). These results should be considered when cord blood gas values obtained at delivery are correlated with those from the prenatal fetal state. Blood gas values obtained at delivery may not reflect the true prenatal fetal acid-base status. (AM J OBSTET GYNECOL 1991;165:1026-9.)

Key words: Cordocentesis, fetal blood gases, delivery cord blood gases.

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Apgar scores and umbilical cord blood gas values have been used separately and in conjunction to define birth asphyxia and to predict newborn outcome. ¹⁻⁴ Umbilical cord blood pH is generally considered to be the most sensitive indicator of birth asphyxia. Thorp et al. ⁵ recently reported on the importance of obtaining um-

Table I. Predelivery and postdelivery cord blood gas values

		Predeliver	y cord values	
Patient No.	рΗ	Pco ₂	PO ₂	Base excess
1	7.37	40.7	34.6	-0.8
2 .	7.31	38.5	44.6	-2.3
3	7.33	49.0	18.0	0.2
4	7.37	40.2	26.9	0.7
4 5	7.42	38.6	39.4	1.1
6	7.38	40.1	45.8	-0.4
7	7.34	41.5	37.2	-1.9
8	7.39	40.0	29.0	0.0
9	7.37	42.8	35.1	0.3
10	7.36	44.3	37.1	0.5
11	7.38	37.8	44.6	-1.1
12	7.32	42.0	22.0	-3.4
13 .	7.33	47.7	19.7	-0.8
14	7.35	43.0	36.7	-0.8
15	7.35	42.1	33.9	-1.4
16	7.40	34.5	28.4	-1.9
17	7.36	36.8	26.7	-1.4
Mean \pm SD	$+7.36 \pm 0.03$	41.15 ± 3.66 *	$32.92 \pm 8.54*$	$-0.79 \pm 1.$

^{*}p < 0.02.

 $[\]dagger p < 0.0003$.

 $[\]ddagger p < 0.0001$.

bilical cord blood gas measurements in all deliveries. The presence of umbilical cord acidemia in the vigorous newborn did not correlate well with neonatal outcome. In their study, 3% of newborns with an umbilical venous pH <7.20 had normal Apgar scores. The cause of acidemia in these vigorous newborns remains speculative.5,6 It may be maternal in origin, as a result of increasing acidosis in the mother as labor progresses, and thus not reflecting true fetal acid-base status.

The introduction of cordocentesis has made it possible to safely study fetal acid-base status in the intact circulation. The use of cordocentesis for fetal blood sampling removes many of the confounding variables that may affect cord blood gas values, such as labor uterine manipulation, anesthesia, and expulsive forces of the mother. The purpose of this study was to compare umbilical blood gas values obtained at cordocentesis with those obtained at delivery and cord clamping.

Material and methods

The study group included 18 healthy pregnant women at term requiring elective cesarean section. Entry criteria included singleton pregnancy, ≥38 weeks' gestation, intact fetal membranes, absent medical or obstetric risk factors, reactive nonstress test before the procedure, and no evidence of labor. All fetuses were structurally normal with appropriate growth. All patients received and signed consent forms previously approved by the investigational review board. Patients were admitted to the labor and delivery suite in the morning, and external fetal monitoring was performed. After absence of uterine contractions and a

	Postdelivery cord values				
рН	PCO_2	Po ₂	Base excess		
7.33	46.9	24.6	-0.7		
7.29	47.4	30.8	-3.2		
7.35	44.8	24.2	-0.2		
7.32	44.8	22.6	-2.0		
7.34	42.7	26.4	-1.0		
7.35	35.6	32.6	-4.6		
7.26	50.4	25.0	-3.8		
7.32	45.8	26.5	-1.5		
7.33	47.0	30.4	-0.5		
7.22	62.5	19.0	-2.7		
7.32	48.0	28.0	-1.2		
7.29	42.1	29.5	-5.4		
7.33	42.5	31.9	-2.7		
7.34	43.6	32.4	-2.3		
7.31	44.7	31.6	-1.9		
7.25	54.0	19.0	-3.9		
7.31	44.1	24.0	-2.0		
7.31 ± 0.04 ‡	$46.29 \pm 5.71*$	$26.97 \pm 4.43*$	$-2.36 \pm 1.48 \dagger$		

reactive heart rate tracing with no abnormalities were documented, each patient was transferred to the operating room and placed in the left lateral decubitus position. An epidural catheter was inserted; however, no medications were introduced into the catheter. A fluid preload of 1000 ml lactated Ringer's solution was administered before initiation of epidural blockade; after blockade was achieved none of the patients had hypotension necessitating treatment with ephedrine or any other vasopressor. Supplemental oxygen was not used until the infant was delivered and cord gases were obtained. Because of the inherent delay from cordocentesis to delivery, the fetal heart was continuously monitored throughout this period, as was maternal blood pressure at 5-minute intervals. The patient was then prepped and draped in the usual sterile fashion for a cesarean section, and cordocentesis was performed by direct ultrasonographic guidance prior to administration of the epidural block.

A 5 MHZ sector transducer was used (ATL Ultramark 5) to locate the placental origin of the umbilical cord when possible; otherwise a free loop of cord was selected. A 5- or 6-inch 22-gauge spinal needle was inserted and guided into the selected portion of the cord. After ultrasonographic confirmation that the umbilical cord was entered, 1 ml blood was collected into prepackaged, preheparinized syringes. Umbilical venous pH and gas measurements were performed within 15 minutes of collection. An additional 3 ml blood was collected into a nonheparinized syringe, and a portion was sent for Kleihauer-Betke smear, to confirm the fetal origin of the sample. The needle was removed and the fetal heart rate was observed for 10 minutes, as was the puncture site, to detect any bleeding or abnormal fetal heart tracing.

The patient was then placed supine with left lateral uterine displacement, and a repeat cesarean section was performed. Within 30 seconds of delivery a segment of umbilical cord was clamped at both ends and venous blood collected into 1 ml heparinized syringes. Blood pH, PO₂, PCO₂, and base excess were immediately measured after the blood sample was collected. The blood gas analyzer used was the Instrumentation Laboratory System 1301 analyzer. The margin of error for the blood gas parameters included pH, 0.01; Pco₂, 1.5; and Po₂, 2.

Neonatal outcome included gestational age, birth weight, and Apgar scores at 1 and 5 minutes. The results of blood gases were compared with Student t test. A p value < 0.05 was considered significant.

Results

There were no cases of fetal bradycardia or fetal heart rate decelerations during or after cordocentesis. The mean \pm SD of observed blood streaming from the umbilical cord was 24 ± 20 seconds (range, 20 to 70 seconds). Fetal blood was documented by Kleihauer-Betke smear in all cases. Seventeen patients underwent epidural anesthesia, and one had general anesthesia because of failed epidural anesthesia. Because of the different mode of anesthesia, this patient was not included in the analysis. There was no significant difference in blood pressure before and after initiation of epidural anesthesia (mean systolic, 130 vs 122 mm Hg; mean diastolic, 82 vs 78 mm Hg). None of the patients had a reduction in either systolic or diastolic blood pressure ≥18 mm Hg. The mean ± SD interval from cordocentesis to delivery was 22.6 ± 2.3 minutes (range, 18 to 30 minutes). Table I compares the blood gas findings at cordocentesis with those at the time of delivery in the 17 patients receiving epidural anesthesia. There were significant differences in all parameters studied between cordocentesis values and delivery cord values. The data were subsequently analyzed according to whether the systolic blood pressure after epidural anesthesia decreased by <10 or ≥ 10 mm Hg. In the group of patients where the change in the systolic blood pressure was <10 mm Hg (n=10), the mean \pm SD of cord pH predelivery was 7.36 ± 0.04 and after delivery was 7.32 ± 0.03 (p < 0.009, by paired t test). In the group of patients where the change in the systolic blood pressure was ≥ 10 mm Hg (n = 7), the mean \pm SD of cord pH predelivery was 7.36 ± 0.03 and after delivery was 7.30 ± 0.04 (p < 0.025, by paired t test). Thus the magnitude of change of the systolic blood pressure did not affect the results of pH values.

All infants were structurally normal and had a birth weight >25th percentile for gestational age. The mean \pm SD for gestational age was 39.6 \pm 0.76 weeks (range, 38.0 to 40.5), for birth weight it was 3419 \pm 467 gm (range, 2900 to 3870). The mean \pm SD Apgar scores were 8.2 \pm 1.0 (range, 6 to 9) and 8.6 \pm 1.4 (range, 7 to 9) for 1 and 5 minutes, respectively.

Comment

With the development of fetal blood sampling by cordocentesis, we are afforded the opportunity to study the fetal acid-base status in the intact fetal circulation. There is a paucity of data on normal blood gas parameters in the term fetus before labor. Previous studies with cordocentesis have provided reference ranges for fetal blood gas parameters. The However, the patient population in these studies came from a high-risk referral group in which the suspected pathologic condition was ruled out by the fetal blood sampling; thus this may not truly represent a normal control group. In addition, there were few patients included with a gestational age >38 weeks.

Unlike previous reports, this study is unique in that it comprised a homogeneous group of patients, devoid of perinatal risk factors and without signs of fetal growth failure. To our knowledge, this is the first report comparing umbilical venous delivery pH in an experimentally controlled fashion with the actual in utero fetal acid-base status. This was possible because we studied healthy pregnant women at term who required elective cesaran section before the onset of labor. Consequently, many of the confounding variables that might affect cord blood gas values were removed, and the interval from fetal sampling to delivery was thus shortened. Although there was still a considerable interval period, it should be noted that the fetal heart and maternal blood pressure were monitored continuously during this period, and no significant changes were noted that may have influenced the results.

The results of this study reveal a significant difference in blood gas values at cordocentesis to those at delivery. The pH and Po2 of the cord blood at delivery were significantly lower than those obtained from fetal blood, whereas the Pco₂ and base excess were consistently higher. These findings may have important implications when relating umbilical cord blood gas results obtained at delivery to the actual prenatal fetal acid base status, and when using these cord blood gas parameters as an end point for evaluating methods of antepartum surveillance. None of the fetuses or neonates studied in this report had evidence of acidosis; however, it is important to note that the average difference in pH values between the two samples was 0.04 units (range, 0.02 to 0.15). This exceeds the margin of error reported for the blood gas analyzer used. Thus it is conceivable that in certain clinical situations an acidotic pH may be present on cord blood at delivery when in fact the fetus is not in an acidotic state.

Most infants with acidosis at birth appear to be in a vigorous or intermediate condition, which raises the possibility that such acidosis is not entirely fetal in origin. Indeed, other authors have postulated that there is movement of hydrogen ions and lactate across the placenta from mother to fetus. Maternal acidosis that takes place during the second stage of labor is to some extent due to the expulsive and ventilatory efforts by the laboring patient during uterine contraction. Consequently, this may result in a downward trend in fetal pH, showing increasing steepness as delivery approaches, due to this net movement of hydrogen ions from mother to fetus.9-18 In addition, the cord blood gas values may be influenced by factors that involve interruption of the uteroplacental circulation at delivery, as well as in the actual collection of the sample for analysis.14

In summary, the acid-base status of the fetus as determined by cord blood gas analysis may not accurately reflect the true acid-base status of the fetus in utero. This may help explain the reported discrepancy between acidosis detected at delivery and neonatal outcome.5,6 These differences in blood gas parameters obtained in utero as compared with those obtained at delivery are most likely related to variables surrounding the delivery process and to techniques of collection and processing of blood samples. However, a larger number of patients will be helpful to ascertain these differences. Finally, the blood gas parameters found at time of cordocentesis in this study may be used as a reference range for the normal term fetus.

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Amniotic fluid bilirubin and fetal hemolytic disease

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Six different methods for assessing amniotic fluid bilirubin were compared in 111 samples from 37 patients. When the Liley methods were compared, the chloroform extraction and 5% correction methods resulted in 20% and 4% reductions, respectively, in the mean change in optical density at 450 nm when compared with the uncorrected mean $(0.086 \pm 0.06 \ [p < 0.05])$ and $0.104 \pm 0.07 \ [p]$, not significantly vs 0.108 ± 0.08). This reduction was observed in spite of significant visual blood contamination being rarely noted. The predictions based on amniotic fluid analysis were compared with the first fetal or neonatal hematocrit. The chloroform-extracted change in optical density at 450 nm accurately predicted fetal status in all patients, whereas lesser degrees of accuracy were observed with other methods. Hydrops fetalis did not occur during the observation period, and fetuses needing transfusion (n = 5) or early delivery (n = 10) were indicated correctly. The chloroform extraction change in optical density at 450 nm accurately predicts fetal status, and its use should continue. (Am J Obstet Gynecol 1991;165:1030-5.)

Key words: Amniotic fluid bilirubin, fetal hemolytic disease

In 1961, to evaluate the severity of fetal hemolytic disease, Liley introduced a technique of amniotic fluid bilirubin analysis that revolutionized the care of patients at risk. The results reported in that article suggested that the severity of hemolytic disease could be predicted by considering the size and trend of the 450 nm spectrophotometric peak in amniotic fluid in relation to the three zones of classification described. Extra caution was recommended to prevent overestimation of the severity of hemolysis when contaminants, such as oxyhemoglobin, were present in the fluid. A technique for the correction of the change in optical density (OD) at 450 nm (ΔOD₄₅₀) (the 5% method) also was described.

In 1986 Nicolaides et al.² assessed amniotic fluid specimens (N = 475) from unaffected fetuses of 16 to 36 weeks' gestation. No method for evaluating or correcting for the presence of oxyhemoglobin was described. A large proportion of values fell into the middle zone of the Liley graph rather than into the unaffected low zone. Among 59 sensitized patients, 25% of whose fetuses were hydropic at the time of sampling, the predictive value of the ΔOD_{450} was determined to be limited. Fetal blood sampling was performed on patients at risk. The authors concluded that the only reliable method for determining the severity of rhesus

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6/6/30895

isoimmunization in the second trimester of pregnancy is the direct measurement of fetal hemoglobin.

The implications of this study's conclusions are enormous. Routine fetal blood sampling of sensitized patients in the second trimester is endorsed. Before such a recommendation can be adopted, careful consideration must be given to the accuracy of the assessment of the Liley technique, the increased technical difficulty and risk of umbilical vein sampling, the lack of widespread availability of the technique, and the potential of routine sampling for worsening the fetal sensitization. These concerns prompted our investigation.

The hypothesis of our study is that the properly performed and carefully interpreted, corrected ΔOD_{450} , assessed for both size and trend of value, will accurately predict the severity of fetal hemolytic disease. Further, if this is so determined, continued use of amniocentesis and ΔOD_{450} offers advantages over routine fetal blood sampling.

Material and methods

The Perinatal Laboratory of the University of Louis-ville Department of Obstetrics and Gynecology is a regional referral site for amniotic fluid analysis. Amniotic fluid specimens from patients at risk for hemolytic disease in the fetus were obtained, largely from ultrasonographically directed amniocentesis performed in our obstetric ultrasonography center. Those specimens were processed immediately. Fluids received from other facilities were sent chilled and light protected.

The specimens were placed in a centrifuge (GCA/Precision Scientific, Chicago) at 1000g for 5 minutes to remove red cells and other debris, and the supernatant was filtered through Whatman No. 4 filter

paper (Whatman International Ltd., Maidstone, England). After centrifugation, the pellet and supernatant were visually inspected for blood or meconium contamination with a continuous recording spectrophotometer (Milton Roy Spectronic 1201, Milton Roy Company, Rochester, N.Y.) with a 1 cm light path quartz cuvette. An aqueous solution of 8-hydroxyquinoline was used as a quality control solution. Specimens with visual evidence of high concentrations of bilirubin were diluted as necessary. Six different methods of bilirubin analysis were performed.

Liley ΔOD_{450} . The specimens were scanned between the wavelengths of 350 and 600 nm on a linear scale against a water blank. Individual readings were taken at 550, 450, and 365 nm. These values were transferred to a semilogarithmic scale, and a straight line was drawn between 550 and 350 nm. The difference between the straight line and the individual point reading at 450 nm, the Liley ΔOD_{450} , was plotted on a Liley graph to assess the severity of hemolysis.1

Chloroform-extraction Liley ΔOD_{450} . Four milliliters of chloroform (Spectranalyzed; Fisher Scientific, Springfield, N.J.) were added to an equal volume of centrifuged amniotic fluid. The mixture was held on a vortex mixer (Vortex Genie 2, Fisher Scientific, Springfield, N.J.) for 30 seconds and placed in a -18° C freezer (White Westinghouse, Dublin, Ohio) for 5 minutes. It was centrifuged for 5 minutes at 1000g, and the bottom chloroform layer was scanned in a recording spectrophotometer. A chloroform blank was used in this procedure.3 The remainder of the analysis was identical to the standard Liley technique.

Five percent corrected Liley ΔOD_{450} . The ΔOD_{410} (oxyhemoglobin peak) was determined by calculating the difference between the straight line (drawn from 375 to 525 nm) at 410 nm and the individual reading at 410 nm. Five percent of this value was calculated, subtracted from the original Liley ΔOD₄₅₀ to produce the corrected $\Delta \mathrm{OD}_{450}$, and plotted and interpreted as 'with the other Liley techniques.'

Queenan ΔOD_{450} . The amniotic fluid samples were scanned between the wavelengths of 350 and 600 nm, recording the absorbance against the wavelengths on a linear scale. A water blank was used to zero the instrument. A straight line was drawn from 375 to 525 nm, demonstrating the approximate course of the amniotic fluid scan in the absence of bilirubin pigments. The difference between the straight line and the graph at 450 nm is the ΔOD_{450} and is linearly proportional to the concentration of bilirubin. The value obtained was plotted on Queenan's prediction graph to indicate the severity of disease.4

Queenan corrected ΔOD_{450} . The absorbance of the curve at 575 nm was subtracted from the absorbance of the curve at 455 nm. Since the alteration of the curve due to the absorbance of oxyhemoglobin at 575 nm is

thought to be essentially the same as that at 455 nm, subtracting corrects for contamination. This value was plotted on Queenan's corrected prediction graph to assess severity of disease.4

Percent transmittance. Centrifuged and filtered amniotic fluid was evaluated with a recording spectrophotometer. The percent transmittance reading at 520 nm was divided by the percent transmittance at 490 nm against a distilled water blank. The resulting value is the percent transmittance ratio.5 The fetus was considered at risk with the following values: percent transmittance ratio ≥ 1.25 before 30 weeks' or a ratio ≥ 1.15 after 30 weeks' gestation.6

The three Liley $\Delta \mathrm{OD}_{450}$ measurements were generally interpreted as previously suggested.^{1, 7} However, consistent with our clinical experience, all management recommendations were based on the chloroform extraction ΔOD_{450} method. Values in the lower zone and the lower one third of the middle zone were considered reassuring, and repeat evaluation was performed within 3 to 4 weeks. Values in the upper zone or in the upper one third of the middle zone were considered ominous and prompted umbilical vein sampling or delivery if pulmonary maturity was demonstrated. Selected patients with values in the upper one third of the middle zone were reassessed by bilirubin analysis within 7 to 10 days. Values in the middle third of the middle zone prompted repeat amniocentesis within 2 weeks. A declining trend was considered reassuring. A level or rising trend was interpreted as possible fetal jeopardy. However, umbilical vein sampling was not performed until the $\Delta \mathrm{OD}_{450}$ was at, or exceeded, the upper third of the middle zone.

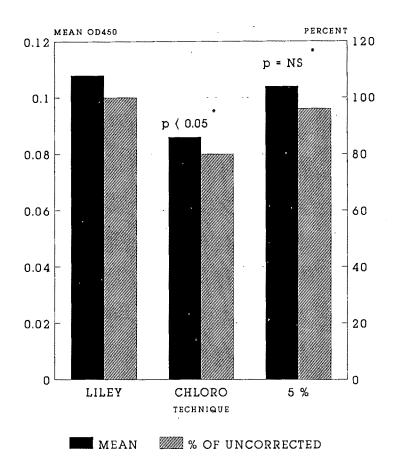
The Queenan values were considered reassuring if in the lower zone, ominous if in the upper zone, and requiring trend analysis if within the area of overlap.4 The percent transmittance ratio was considered reassuring or nonreassuring as described above.

The following outcome parameters were evaluated: (1) estimated gestational age at delivery, (2) first umbilical vein hematocrit at delivery or percutaneous sampling, (3) neonatal direct 'Coombs' test values, and (4) neonatal length of hospitalization. The accuracy of each method of bilirubin analysis was determined for each patient. The results were categorized as accurate, accurate but requiring trend analysis, or inaccurate (misleading).

Differences among the means were analyzed by analysis of variance. Differences between group means were evaluated by the least significant difference t test. Significance was set at p = 0.05.

Results

From June 1988 through June 1990, 111 amniotic fluid specimens were obtained from 37 patients (3.0 samples per patient; range I to 6). Forty-four speci-



103 SAMPLES; * versus uncorrected mean

Fig. 1. Mean ΔOD₄₅₀ among three Liley techniques, uncorrected (*LILEY*), chloroform extraction (*CHLORO*), and 5%. For each method percent of uncorrected mean is represented. NS, Not significant.

mens were obtained from patients at or before 29 weeks' gestation. Nineteen patients were antibody positive for the Rho(D) antigen (either alone or in combination). The remaining patients were sensitized to single or multiple atypical antigens. The mean gestational age at amniocentesis was 29.5 weeks (range 21 to 39 weeks).

Inspection of the spectrophotometric curve at 410 nm (oxyhemoglobin peak) demonstrated a measurable, positive deviation from expected for 86 of 111 (77%) samples, resulting in a downward correction of the $\Delta \mathrm{OD_{450}}$ (5% correction method) for these samples. Similarly, the chloroform extraction method resulted in a decrease of the $\Delta \mathrm{OD_{450}}$ in 93 of 103 (90%) specimens. These corrections were found in spite of visually noting only 32% (35/111) of specimens were contaminated by blood and finding, of those, only 66% (23/35) with trace evidence of blood (<1%) in the centrifuged pellet. Meconium and concentrated bilirubin were each noted in only one specimen.

Among ΔOD_{450} values in which all three Liley methods were assessed (n=103), the chloroform extraction

and 5% correction methods, respectively, resulted in a 20% (0.086 \pm 0.06; p < 0.05) and 4% (0.104 \pm 0.07; p, not significant) reduction in the mean ΔOD_{450} when compared with the uncorrected value (0.108 \pm 0.08) (Fig. 1).

There were no known perinatal losses. Hydrops fetalis did not occur. Umbilical vein sampling was required in only five of 37 patients (14%). Two fetal hematocrits were <15% (9% and 13%) without evidence of hydrops. In both cases the first amniocentesis and umbilical vein sampling were performed immediately on entry into care. The other three first fetal hematocrits were 17.3%, 17.5%, and 34.5%. The neonatal findings of the 25 patients whose infants were noted to have a positive direct Coombs' test are summarized in Table I. Of the remaining 12 patients, one underwent intraperitoneal transfusion and fetal hematocrit was not available, three neonates were known to be direct Coombs' negative, six were noted to have low-zone ΔOD₁₅₀ values at 34 to 37 weeks' gestation and specific follow-up was not obtained, and two patients were lost to follow-up. Unexpected severe disease, as measured

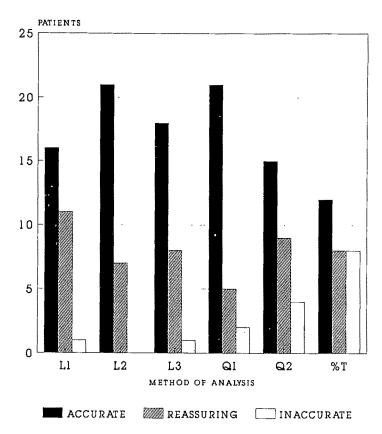


Fig. 2. Comparative accuracy of each method, uncorrected Liley (L1), chloroform extraction (L2), 5% corrected (L3), Queenan (Q1), corrected Queenan (Q2), and percent transmittance (%T), among 28 patients with known outcomes. Designation of "accurate" was given if each individual amniotic fluid value correctly identified fetal status. "Reassuring trend" was assigned if declining trend of values (two or more amniocenteses) was apparent. "Inaccurate" was assigned if method was inappropriately reassuring or alarming.

by first hematocrit, was not observed. The first hematocrit ranged from 33% to 66% among newborns who did not receive fetal transfusion.

The predictions of the six methods of bilirubin analysis used in the 28 patients with known outcomes are summarized in Fig. 2. The chloroform-extracted ΔOD₄₅₀ correctly identified fetal status at each sampling performed in 21 of 28 patients. The remaining seven patients had results in the middle third of the middle zone but had a downward trend (reassuring) in each case (Fig. 3). Four of these patients' values dropped into the lower zone at third-trimester sampling. Thus trend analysis was required more frequently in the second trimester. No patient was inaccurately categorized. Four of the five fetuses who underwent umbilical vein sampling were noted to have hematocrits of <20% without evidence of hydrops fetalis. If used for clinical management, each of the other techniques would have either required more trend analysis for reassurance or prompted unnecessary fetal blood sampling. Queenan's uncorrected method performed nearly as well as the chloroform extraction technique. The 5% method

Table I. Neonatal outcomes among infants (n = 25) with positive results on direct Coombs' test

	Mean ± SD	Range
Estimated gestational age (wk)	36.1 ± 3.2	29-41
Birth weight (gm)	2852 ± 688	1446-4281
First hematocrit (%)	42.0 ± 11.4	16.6-66.6
First hematocrit (%)*	46.4 ± 7.5	33.9-66.6
Apgar score		
l min	7 ± 2	1-9
5 min	8 ± 1	5-9

^{*}Excluding transfused fetuses (n = 20).

did not reduce the ΔOD₄₅₀ as much as the chloroform extraction technique and resulted in more samples falling in the middle of the middle zone, prompting more frequent amniocentesis if used clinically. The percent transmittance technique did not discriminate accurately enough between fetuses requiring or not requiring blood sampling. In other words, when compared with the other techniques, the chloroform extraction tech-

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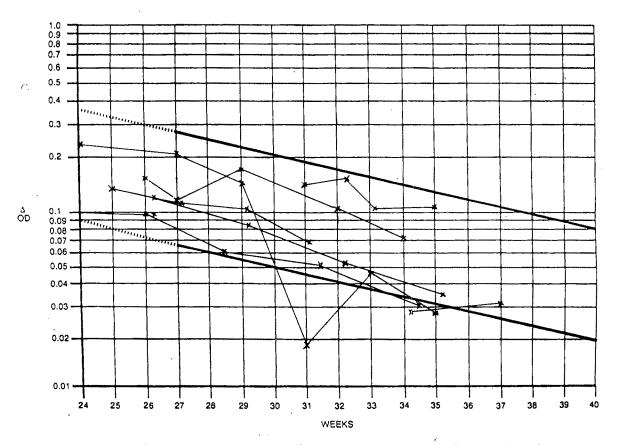


Fig. 3. Chloroform-extraction ΔOD_{450} values for seven patients who required trend analysis for management decisions.

nique allowed a more accurate interpretation of fetal status and permitted less frequent amniocentesis.

Comment

The findings of this study are noteworthy for several reasons. The influence of amniotic fluid contamination by blood, from either a maternal or fetal source, may be greater than previously appreciated. Although visual evidence of trace or greater contamination was noted in 32% of the centrifuged pellets of amniotic fluid specimens, direct evaluation of the curve by the 5% method resulted in a reduction of the ΔOD_{450} for 77% of samples by an average of 4%. More striking was a 20% reduction of the mean $\Delta \mathrm{OD}_{450}$ by the chloroform extraction technique. This percent reduction is similar to the results reported originally.3 Perhaps visual inspection alone is inadequate to decide whether correction is necessary. We can only speculate why there is such a substantial difference between the correction techniques. The 5% correction of Liley' may merely undercorrect the value. The original description suggests the method was based on experimental contamination; however, no details of the findings were given. The chloroform extraction technique has been noted to successfully recover all bilirubin when compared with preextraction values,3 so artifactual lowering of the bilirubin content seems unlikely.

Our patient management was based entirely on the chloroform extraction ΔOD_{450} method. Patients required an average of three amniocenteses. There were no fetal deaths, and hydrops fetalis did not occur under our supervision, despite infrequent use of umbilical vein sampling. There were no surprise severe anemias. Among fetuses thought to have severe anemia who underwent blood sampling, four of five were severely anemic and were at or near values associated with hydrops fetalis. While hydrops did not occur, sampling was not performed until bilirubin values were in the upper zone of Liley's graph (two were at first amniocentesis). As a consequence, we have become more aggressive when high or rising midzone values are noted. Only one first fetal umbilical vein hematocrit (34.5%) was above that which prompts fetal transfusion.

Nicolaides et al.² noted that most ΔOD₄₅₀ values among the 475 samples collected from nonsensitized patients fell in the middle zone of the Liley extended graph. The graph used in that study is an arbitrary, not data-based, extension of Liley's original graph.⁸ No correction technique was identified as having been performed on the samples. From visual inspection of data points, it would appear that many, if not most, of the values would have been relocated in the low zone or in the lower third of the middle zone if an average 20% correction had been applied to the noted values. Thus

most of the values would fall in a reassuring range. Few details regarding these patients were reported; therefore the conditions that prompted sampling (viral disease, fetal anomaly) possibly could have altered amniotic bilirubin, independent of red blood cell sensitization.

In the above-mentioned report,2 the authors assessed the interpretability of the uncorrected ΔOD_{450} method among sensitized patients. Included in the patient sample were 25 (of 59) patients whose fetuses were hydropic at the time of sampling. We believe it is inappropriate to include such patients in this analysis. Most clinicians would consider an amniocentesis unnecessary when hydrops had been noted. In such cases it is possible that fetal anemia may be so profound that amniotic fluid bilirubin is lowered because of lack of substrate (fetal red blood cells). In addition, associated polyhydramnios may exert a diluting effect on optical density. In spite of this, we noted that only one of the 25 hydropic fetuses (no details given) had a ΔOD₄₅₀ value below the middle of the middle zone. Further, the authors did not attempt to evaluate the trend of serial samples but merely judged the individual values. Trend analysis, as first emphasized by Liley,1 is necessary to properly interpret values in the middle zone. We needed to use trend analysis for seven of the 28 patients in this study; its use safely and successfully avoided unnecessary umbilical vein sampling. Trend analysis may be required more frequently in the second trimester. However, this may be the consequence of an inaccuracy in the arbitrary extension of the Liley graph. A data-based modification of the extended Liley graph using chloroform-extracted values is probably warranted. However, because of uncertainty regarding the technique used and patients enrolled in the study of Nicolaides et al.,2 we believe his data may not suit this purpose.

Nicolaides et al.² have concluded that the only reliable method for second-trimester evaluation of patients at risk is the direct measurement of fetal hemoglobin. We argue that our experience supports the continued use of amniotic fluid analysis in the second trimester. Their assessment of amniotic fluid analysis by the Liley method is flawed by a failure to correct for fluid contamination, inclusion of hydropic infants in the analysis, failure to judge trend analysis, and no assessment of the risk/benefit ratio for routine fetal blood sampling versus amniotic fluid analysis. A consensus estimate of fetal loss from umbilical vein sampling, although difficult to extract from the literature, is probably 1% to 2%.9. 10 Umbilical vein sampling, particularly when transplacental, may worsen the severity of sensitization as much as, if not more than, amniocentesis. The appropriate interval between blood sampling episodes has not been adequately studied. Does a fetal hematocrit

of 33% prompt reevaluation in 2 or 3 or 4 weeks? Will routine second-trimester blood sampling, if used, interfere with third-trimester amniotic fluid analysis? Will pregnancy outcome be improved by this more hazardous technique? The authors offer no real comparative data on which they base their conclusion.

The American College of Obstetricians and Gynecologists, apparently in response to the article by Nicolaides et al.,2 has revised its technical bulletin11 regarding isoimmunization. In the October 1990 bulletin, the Liley graph is presented without the second-trimester extension previously endorsed in the January 1986 bulletin.8 Fetal blood sampling is recommended for most patients at risk first seen in the second trimester. The willingness of the American College of Obstetricians and Gynecologists to change its recommendations on the basis, in large part it would appear, of this one report is surprising.

For now, an endorsement of routine fetal blood sampling to assess patients at risk is premature and inadvisable. Properly performed and interpreted amniotic fluid bilirubin analysis by the chloroform extraction technique permits accurate fetal assessment, thereby in most cases (86% in our series) avoiding fetal blood sampling.

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What is a low-lying placenta?

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Transvaginal ultrasonography was performed in 127 women thought to have placenta previa. In all cases of complete previa, placental location was confirmed at cesarean section. Where the placenta was situated in the lower segment of the uterus but did not cover the cervical os the distance from the placental edge to the internal cervical os was measured. This distance was analyzed in relation to the route of delivery. No patient with a placental edge >2 cm from the internal cervical os required cesarean section for the indication of placenta previa, whereas seven of eight patients with a distance of ≤2 cm underwent cesarean section because of bleeding characteristic of a placenta previa. These preliminary results suggest that transvaginal ultrasonography measurement may indicate the optimal delivery route and make the traditional classification of placenta previa obsolete. (AM J OBSTET GYNECOL 1991;165:1036-8.)

Key words: Placenta previa, transvaginal ultrasonography, cesarean section

The classification of placenta previa into complete, partial, marginal, and low-lying was originally based on digital palpation of the placenta through the cervical os1 and has remained unchanged in spite of the advent of modern obstetric care and the widespread use of ultrasonography for the diagnosis of placenta previa.2 This is at least in part because the standard method of transabdominal ultrasonography is often insufficiently precise in determining the exact relationship of the placental edge to the internal cervical os.⁸ The greater resolution of transvaginal ultrasonography enables accurate visualization of the internal os.4 Transvaginal ultrasonography has been shown to be a safe technique in placenta previa and to be superior to transabdominal ultrasonography in the prediction of placental location at delivery.⁵⁻⁷ Where the placental edge encroaches on the os, the intervening distance can be measured at transvaginal ultrasonography. In this study we retrospectively examined the role of a classification of placenta previa with the use of transvaginal ultrasonographic measurement in predicting the route of delivery.

Material and methods

One hundred twenty-seven women with antepartum hemorrhage or with previous transabdominal ultrasonography suggestive of placenta previa were referred for further ultrasonographic evaluation. All patients

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underwent transabdominal ultrasonography and transvaginal ultrasonography. Transvaginal ultrasonography was performed with the use of a 5.5 MHz vaginal probe (Acuson, Mountain View, Calif.) after the patient voided. Placenta previa was defined as any placental tissue visible in the lower segment of the uterus at transvaginal ultrasonography. A complete previa was diagnosed if placental tissue extended over the internal cervical os. Where a placental edge was visible on transvaginal ultrasonography but did not cross the internal os, the intervening distance was measured in centimeters by a minimum of two operators (Fig. 1) and a mean value was calculated. Only the results of ultrasonographic scans performed in the third trimester were included in the study, and where ultrasonography had been repeated, the most recent studies were analyzed. The outcome measure was route of delivery, with verification of placental location if delivery was by cesarean section. All ultrasonographic results were available to the physicians, who made the decision to perform cesarean section on the basis of the presence of significant bleeding.

Statistical significance was calculated with the unpaired t test.

Results

Transvaginal ultrasonography was performed in 127 patients at an average gestational age of 33 ± 4 weeks (mean \pm SD) and delivery occurred at 38 ± 3 weeks. Of the 127 patients, 52 were diagnosed as having placenta previa. The internal cervical os was visualized at transvaginal ultrasonography in all cases. Thirty-one patients had complete previa, and the placental location was confirmed at subsequent cesarean section in all these cases. In the remaining 21 cases the outcome variable of mode of delivery was correlated with the

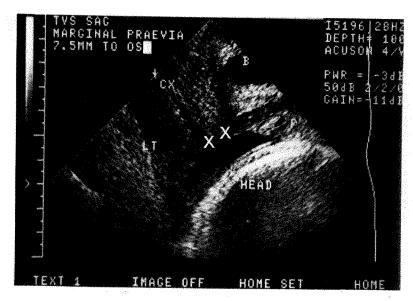


Fig. 1. Transvaginal ultrasonographic scan at 34 weeks' gestation. Cervical canal is clearly visible (CX) and distance from internal os to placental edge, measured between calipers (X), is 0.75 cm. The patient was delivered by cesarean section 4 weeks later because of vaginal bleeding. P, Placenta; B. bladder.

transvaginal ultrasonographic distance from the placental edge to the internal os. In 7 of these 21 patients who required a cesarean delivery because of bleeding, the mean distance was 1.1 ± 0.8 cm (range, 0 to 2 cm), compared with a distance of 3.1 ± 1.1 cm (range, 1.8to 5.8 cm) in the remaining 14 patients who did not require a cesarean section because of placenta previa (p = 0.0004). Four patients in the latter group underwent cesarean section for other reasons and 10 were delivered vaginally. The differences in the distances from the placental edge to the internal os between these two groups were statistically significant (p = 0.0004).

Only one patient with a placental edge within 2 cm of the cervical os was delivered vaginally. In this case transvaginal ultrasonography had been performed at 28 weeks, and delivery occurred 11 weeks later.

There were three cases in this series in which a "double setup" examination was performed. In two of these the placental edge was within 2 cm of the cervical os by transvaginal ultrasonography and cesarean section was performed. In the third case the placental edge lay 5.5 cm away and the patient was delivered vaginally.

Comment

The terminology of the traditional classification of placenta previa was derived from digital palpation of the placenta through a partly dilated cervix in labor. There is little debate about the diagnosis and management of a complete previa in late pregnancy, but the terms partial and marginal placenta previa are not applicable to the antenatal ultrasonographic appearance of the cervix, which is visualized as a discrete point.8.9

Similarly, the concept of the low-lying placenta provides little information about its clinical significance. In those cases where the placenta does not cover the os antenatally, what is really required from ultrasonography is a prediction of the likely clinical course and mode of delivery, on the basis of actual distance between the placental edge and the internal cervical os.

A change in the relative position of the placenta, or placental migration, can occur in the third trimester, 10 and this may explain the one case in our series with a placental edge within 2 cm of the os in which vaginal delivery occurred. It is now our practice to perform transvaginal ultrasonography every 4 weeks in such cases to document migration. In the three cases where double setup examination was performed, the transvaginal ultrasonographic distance from the placental edge to the internal os would have predicted whether the placenta would be palpable on digital examination. Therefore transvaginal ultrasonography could replace the requirement for the double setup examination.

It is possible that the transvaginal ultrasonographic result itself influenced management decisions; however, we suggest that the greater accuracy of transvaginal ultrasonography in excluding complete previa encouraged the physician to manage these cases expectantly. There is no absolute gold standard for the diagnosis of placenta previa, and whereas successful vaginal delivery excludes clinically significant previa, the performance of a cesarean section does not prove that vaginal delivery could not have occurred. Although the outcome measure used in this study is not an absolute one, it does reflect a prevailing clinical standard with which to compare the ultrasonographic results.

In conclusion, we have shown that a transvaginal ultrasonographic measurement of placental edge to internal os of >2 cm reliably predicted safe vaginal delivery. A shorter distance was often associated with bleeding necessitating cesarean section. However, further studies are necessary to validate this value as a useful cutoff point. Furthermore, we suggest that the advent of high-resolution transvaginal ultrasonography makes the traditional classification of placenta previa obsolete.

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Pulmonary-to-aorta diameter ratio in the normal and abnormal fetal heart

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In an attempt to establish the normal ratio of pulmonary artery to aorta diameters at varying gestational ages, the pulmonary artery and aorta diameters of 316 normally grown fetuses between 14 and 39 weeks' gestational age were measured. The ratios for each fetus were derived, and regression analysis was used to evaluate the relationship between gestational age and each diameter. We conclude that the diameters of the pulmonary artery and aorta are closely related to fetal age but that the ratio is independent of age (mean, 1.09; SD, 0.17). The diameters of the pulmonary artery and aorta in 21 fetuses with proved congenital heart disease were then compared with those of this normal population. The pulmonary artery/aorta ratio was abnormal in 13 of 21 fetuses with congenital heart disease. Actual measurements of the great vessels can be difficult and may be misleading, but a quick comparison of relative size by inspection is feasible. (AM J OBSTET GYNECOL 1991;165:1038-44.)

Key words: Heart (fetal), pulmonary artery (fetal), aorta (fetal), congenital heart disease (fetal)

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6/6/31429

The current screening view of the fetal heart is the four-chamber view. However, this view does not detect abnormalities involving the great vessels, such as truncus arteriosus, transposition, or double-outlet right ventricle, nor does it always detect tetralogy of Fallot. As examiners become more experienced, the screening examination will undoubtedly evolve to include an eval-

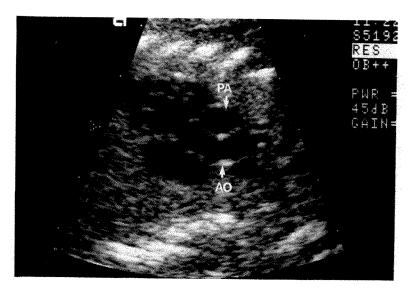


Fig. 1. Long-axis view shows aorta (AO) and pulmonary artery (PA). Measurement of aorta would be obtained at level of arrowheads (inner-to-inner). Pulmonary artery would not be measured from this view because it may not be at level of valve.

uation of the pulmonary artery and aorta in an attempt to detect these additional heart defects. In addition, a glimpse of the great vessels is sometimes possible when an adequate four-chamber view cannot be obtained because of technical factors such as fetal age, motion, or lack of fluid.

Since the size of the pulmonary artery and aorta reflects the relative flows through the right and left sides of the heart, an abnormal relative size of either vessel may imply disturbed flow associated with an underlying congenital heart defect. Although measurements of each individual vessel can be obtained, we have observed that in some fetuses the great vessels seemed disparate in size in spite of the fact that the measurement of each vessel was by itself normal or near normal. This study was an attempt (1) to establish the normal size relationship (pulmonary artery/aorta) at varying fetal ages, (2) to determine whether that relationship is age dependent, and (3) to determine whether it could contribute additional information to the evaluation of the fetal heart.

Methods

The pulmonary artery and aorta diameters were measured at the level of their respective valves from inner wall to inner wall in each of 316 fetuses from 14 to 39 weeks' gestational age from February to November 1990. Measurements were taken in real-time B-mode in either systole or diastole, because it has been previously shown that there is very little variation in size during the cardiac cycle.' A view showing the vessel longitudinally along with the valve itself was deemed acceptable (usually the short-axis view for the pulmo-

nary artery and the long-axis for the aorta, Fig. 1). No measurements were made in cross-sectional views of the vessel in question. Only those fetuses who were normally grown for dates (femur length, estimated fetal weight, abdominal and head circumference, and head circumference/abdominal circumference ratio between the 5th and 95th percentiles) and in whom both vessels could be measured were included. All fetuses with any ultrasonographic anomaly other than mild hydronephrosis or single umbilical artery were excluded from this normal group. The diameters of the aorta and pulmonary artery were plotted against estimated gestational age rather than estimated fetal weight, because it has been previously demonstrated that they are more closely related to age than fetal weight. Linear regression analysis was used to examine the relationship between estimated gestational age and pulmonary artery and aorta diameter. The 5th and 95th percentiles for the normal population were calculated from 14 to 39 weeks. A similar analysis was performed for the pulmonary artery/aorta ratio of each fetus, yielding the mean value and the upper and lower limits of normal (95th and 5th percentiles) for the normal population.

The pulmonary artery and aorta diameters of 21 fetuses with congenital heart disease were obtained retrospectively from our records. Nineteen of these 21 were identified in the antenatal period. Most were incidental findings during our routine four- and fivechamber views of the heart on all fetuses ≥15 weeks. However, several were referred because of suspected anomalies. Thirteen were diagnosed in the 10-month period during which normal measurements were made. but eight had been identified in the prior year. Only

Fig. 2. Pulmonary artery diameter versus fetal age. Pulmonary artery = 0.0313 estimated gestational age (EGA) - 0.235. There is close correlation with age (r = 0.94).

fetuses in whom there were simultaneous measurements of both great vessels and in whom there was autopsy or neonatal confirmation were included.

Results

Pulmonary and aorta diameters increase with gestational age in the normally grown fetus (r = 0.94) (Figs. 2 and 3). The mean pulmonary/aorta ratio is 1.09, with a range (± 2 SD) from 0.75 to 1.43 (Fig. 4). This ratio appears to be independent of fetal age. Beyond 20 weeks' gestational age there were no normal fetuses with a ratio <0.75.

The pulmonary artery/aorta ratio was <0.75 in the four fetuses with tetralogy of Fallot. Conversely, it was normal in four fetuses with uncomplicated ventricular septal defects (Fig. 5).

The pulmonary artery/aorta ratio was high in fetuses with tricuspid atresia with transposition, coarctation, double-outlet right ventricle, hypoplastic left heart, and an interrupted aortic arch. It was low in fetuses with tricuspid atresia and tetralogy of Fallot (Fig. 6).

There were ten sets of measurements in seven fetuses with congenital heart disease in which one vessel was normal-sized and the other normal or just beyond the normal range but in which the pulmonary artery/aorta ratio was very abnormal: coarctation, n=1; hypoplastic left heart, n=1; tricuspid atresia, n=2; and tetralogy of Fallot, n=3.

The positive predictive value was 33%, the negative predictive value 98%, sensitivity 54%, and specificity 96% on the basis of 13 cases of cardiac heart disease discovered during the period in which normal measurements were obtained. Eight cases of cardiac heart disease were detected before that period and are not included in these calculations.

Comment

Two other studies have evaluated the relationship between the size of each of the great vessels and either fetal weight or gestational age. Sahn et al.³ found a weak correlation between the size of the pulmonary artery and aorta and fetal weight (pulmonary artery, r=0.68; aorta, r=0.66). Our data relating pulmonary artery and aorta diameters to gestational age (rather than weight) closely approximate those of Cartier et al.,¹ in which size of the great vessels was found to be significantly related to age in 406 fetuses (pulmonary artery, r=0.96; aorta, r=0.94). The same group found that the aorta and pulmonary artery diameters continue to enlarge at age-approximate rates even if the fetus is growth retarded, confirming a relation to age rather than weight.²

Two smaller studies also have evaluated the actual ratio of the sizes of the pulmonary artery to aorta in individual fetuses. In a two-part study Angelini et al. found that, although the fetal pulmonary artery was

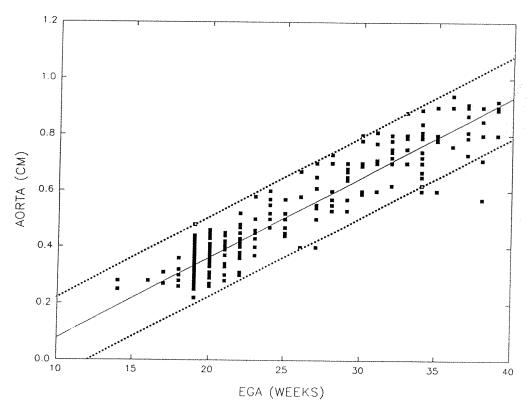


Fig. 3. Aorta diameter versus fetal age (r = 0.94).

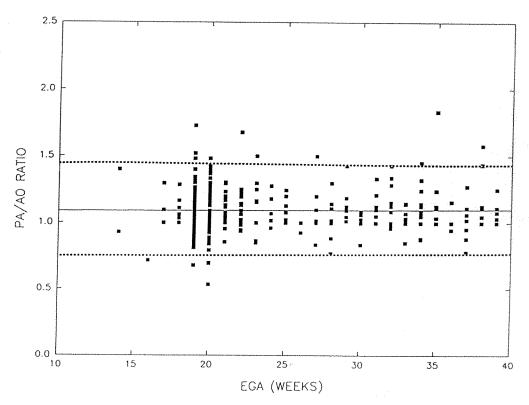


Fig. 4. Pulmonary artery/aorta (PA/AO) ratio versus age (EGA). Mean is 1.09 (SD = 0.17) and range (± 2 SD) is 0.75 to 1.43. This ratio is independent of fetal age.

Fig. 5. Pulmonary artery/aorta (PA/AO)-ratio in fetuses with tetralogy of Fallot (\blacksquare) versus ventricular septal defect (\bullet) and atrioventricular septal defect (\bullet). Mean = 1.09; 50 = 0.17. EGA, estimated gestational age. Dotted lines connect measurements in same fetus.

always larger than the aorta in 20 fetal echocardiograms, in postmortem fetuses the pulmonary trunk was larger than the aorta in 24 cases, equal in size in five, and smaller in one. More specifically, Sahn et al.³ found the pulmonary artery/aorta ratio to have a mean of 1.18 (vs 1.09 in our study) and to be independent of fetal weight in 69 fetuses with estimated weights of 500 to 3400 gm.

We found the mean pulmonary artery/aorta ratio to be slightly less (1.09) and confirm with a larger group (n=316) that it is independent of fetal age. Unlike Angelini et al., we did find that the aorta was larger than the pulmonary artery (pulmonary artery/aorta <1.0) in some normal fetuses (all values <1.0 on Fig. 4). However, in no normal fetus with a gestational age > 20 weeks was the ratio <0.75. The pulmonary artery/aorta ratio was >1.43 in only seven normal fetuses with a gestational age of >20 weeks (i.e., the pulmonary artery was rarely >50% larger than the aorta).

The pulmonary artery/aorta ratio was abnormal in 13 of 21 fetuses with congenital heart disease and normal in eight. As expected, not all fetuses with congenital heart disease have an abnormal ratio; it was normal in fetuses with ventricular septal defects, in a fetus with a ventricular septal defect and a straddling tricuspid valve, and in all fetuses with atrioventricular septal defects. It should be noted that in each of these defects

the blood flow patterns in the pulmonary artery and aorta would be normal in utero. However, the pulmonary artery/aorta ratio did effectively discriminate between four fetuses with ventricular septal defects and four with tetralogy of Fallot.

In fact, in seven cases in which one vessel was normal-sized and one measured near the outer limits of normal, the ratio accentuated the abnormal relationship in a manner similar to that in which the head/abdominal circumference accentuates the abnormal relationship of two measurements that may each be within normal range (tricuspid atresia, 2/2 cases; hypoplastic left heart, 1/1; and coarctation 1/1). This was particularly true in three cases of tetralogy of Fallot in which the aorta was only slightly large (n = 2) or normal in size (n = 1), the pulmonary artery diameter was normal, and yet the pulmonary artery/aorta ratio was abnormal. Since the ventricular septal defect in these fetuses can be difficult to see, comparison of vessel size may be the first suggestion that an abnormality exists.

Evaluation of the size relationship of the pulmonary artery to aorta is not intended to replace actual measurement or Doppler interrogation of the great vessels in the evaluation of the suspected abnormal fetal heart. However, because of position, shadowing by ribs, lack of fluid or fetal motion, Doppler data or even an adequate four-chamber view of the heart is not always

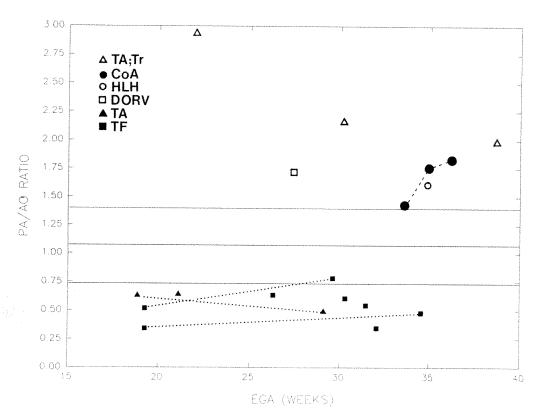


Fig. 6. High pulmonary artery/aorta (PA/AO) ratios occur in fetuses with increased pulmonary artery flow and decreased aortic flow. Mean = 1.09; SD = 0.17. △, Tricuspid atresia with transposition; ●, coarctation or interrupted arch; □, left hypoplastic heart; □, double-outlet right ventricle. Low pulmonary artery/aorta ratio is found in fetuses with decreased pulmonary artery flow and increased aortic flow. ■, Tetralogy; △, tricuspid atresia; EGA, estimated gestational age.



Fig. 7. Aorta is obviously much larger than pulmonary artery in this fetus with tetralogy of Fallot (short-axis view). In fact, pulmonary artery/aorta ratio is 0.5, which is well below 2 SD below normal. PA, Pulmonary artery; AO, aorta.

obtainable. In addition, Doppler measurements of velocity are often time-consuming and therefore not a practical part of a screening examination. Actual measurement of the vessels also can be difficult and may

be misleading if one measurement is high-normal and the other low-normal. A quick comparison of relative size by simple inspection is feasible during the course of a screening examination, because the pulmonary artery and aorta cross each other near their origins. If the pulmonary artery appears to be >50% larger or >25% smaller than the aorta (Fig. 7), a closer examination of the heart is suggested. This ratio has the added advantage of being useful even when fetal age is unknown, because it appears to be independent of age and weight.

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Transverse cerebellar diameter: A useful predictor of gestational age for fetuses with asymmetric growth retardation

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There have been conflicting reports regarding the ability of cerebellar diameter to satisfactorily predict the gestational age of growth-retarded fetuses. Gestational age prediction intervals were derived from 270 normal fetuses between 14 and 40 weeks' gestation for biparietal diameter, head circumference, abdominal circumference, femur length, and transverse cerebellar diameter. We evaluated the ability of these parameters to predict gestational age for 19 small-for-gestational-age fetuses. The cerebellar diameter regression model led to the smallest differences between observed and predicted gestational age for all growth-retarded fetuses. Transverse cerebellar diameter satisfactorily predicted gestational age for all six fetuses with asymmetric intrauterine growth retardation and was associated with the least amount of underestimation bias when compared with other ultrasonographic parameters. However, transverse cerebellar diameter appeared to be no better than biparietal diameter, head circumference, or femur length for accurately predicting gestational age of fetuses with symmetric intrauterine growth retardation (n = 13) despite the finding that cerebellar growth was also relatively spared under these circumstances. We conclude that transverse cerebellar diameter can be used to reliably approximate gestational age in fetuses with asymmetric intrauterine growth retardation. However, caution is warranted when using it to predict the gestational age of fetuses affected by symmetric intrauterine growth retardation. (Am J Obstet GYNECOL 1991;165:1044-50.)

Key words: Cerebellum, intrauterine growth retardation, ultrasonography, gestational age

Physicians caring for pregnancies complicated by intrauterine growth retardation (IUGR) are sometimes faced with a dilemma when patients are seen for the

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first time during the second or third trimester with confusing gestational dating criteria. Early ultrasonographic information is not always available to verify gestational age on the basis of menstrual history alone. A significant correlation between transverse cerebellar diameter and gestational age has been previously reported, but the actual relationship between these two variables remains controversial for growth-retarded fetuses. We have observed some small-for-gestationalage (SGA) fetuses to have transverse cerebellar diameter measurements that were minimally affected by IUGR. Therefore the potential application of such

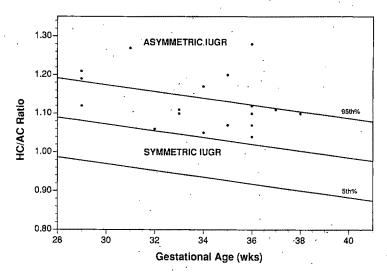


Fig. 1. Head circumference/abdominal circumference (HC/AC) ratio prediction intervals (95th percentile) for 270 normal fetuses.

measurements to reliably estimate gestational age from SGA fetuses was systematically investigated.

Material and methods

Normal population. Obstetric ultrasound scans at William Beaumont Hospital were retrospectively examined over a 2-year period. Ultrasound data were obtained from 270 normal fetuses between 14 and 40 weeks' gestation by Acuson 128 (Mountain View, Calif.), General Electric RT 3000 (Milwaukee), and Aloka 650 (Corometrics, Wallingford, Conn.) imaging systems. Standard measurements included biparietal diameter, head circumference, abdominal circumference, femur length, and transverse cerebellar diameter. Transverse cerebellar diameter measurements were obtained from the outer to outer margins of the cerebellum in the posterior fossa. All fetuses had excellent ultrasonographic dating criteria before 20 weeks' gestation and were found to be subsequently normal by neonatal examination. Gestational ages were rounded off to the nearest whole week. Fetuses with structural abnormalities were excluded, and all infants were delivered at our institution. This information was used to construct gestational age prediction intervals for our normal patient population.

SGA fetuses. Nineteen SGA newborns with birth weights ≤10th percentile of Brenner et al.⁵ were entered into the study from Aug. 3, 1987, to Dec. 11, 1990. Approximately 24,000 ultrasonographic examinations were conducted during this time period. All infants had normal early ultrasonographic scans before 20 weeks' gestation or an initial ultrasonographic examination before 24 weeks that was consistent with last menstrual period. These subjects also required at least one transverse cerebellar diameter measurement after 26 weeks' gestation that could be correlated with other

ultrasonographic findings. Infants with structural or genetic abnormalities were excluded from the study.

SGA fetuses were subdivided according to whether the observed head circumference/abdominal circumference ratio was within our normal population, as defined by 95th percentile prediction limits. The head circumference/abdominal circumference prediction limits were used to classify IUGR types as symmetric (values within normal prediction limits) or asymmetric (values outside of prediction limits). The last ultrasonographic examination before delivery was plotted against these normal curves to qualitatively demonstrate the ability of the regression model to predict gestational age for small fetuses. The mean difference between observed and predicted gestational age values were averaged for each ultrasonographic variable to allow quantitative comparisons between groups. All fetuses, with the exception of one case, delivered at our

Statistical and graphic analysis. Regression analysis and 95th percentile prediction intervals were calculated by JMP Macintosh software for our normal population (SAS Institute, Cary, N.C.).6 Prediction limits were designed to predict gestational age for a given ultrasonographic measurement (e.g., cerebellar diameter). This approach considers the individual variability for a given measurement. By contrast, confidence intervals compare mean values (population vs sample) and do not reflect the variability associated with a single observation. Accordingly, a value that falls within the 5th and 95th percentile prediction limits will not be significantly different from the normal population. Mean values are reported with their standard deviations. Differences between group mean values were analyzed with the Student t test.

Table I. Description of 19 fetuses with IUGR

Case No.	Delivery gestational age (wk)	Birth weight (gm)	Brenner 10th percentile (gm)	Weight difference (%)	Head circumference/abdominat circumference
Asymmetric IUGR					
ĺ	29	879	890	1	1.19
2	35	1560	1870	17	1.17
2 3	31	985	1180	17	1.21
4	30	656	1030	36	1.27
5	36	1956	2190	11	1.23
6	36	1350	2190	38	1.28
Mean values	33 ± 3	1231 ± 482		21 ± 15	1.22 ± 0.04
Symmetric IUGR					
7	36	2110	2190	4	1.06
8	38	2380	2510	5	1.07
9	38	2330	2510	. 7	1.05
10	37	1520	2310	34	1.11
11	39	2320	2680	13	1.07
12	37	1980	2310	14	1.10
13	39	2210	2680	18	1.04
14	35	1875	1870	0	1.10
15	33	1475	1480	0	1.11
16	40	2440	2750	11	1.10
17	40	2170	2750	21	1.10
18	40	2640	2750	4	1.07
19	40	1900	2750	31	1.12
Mean values	38 ± 2	2104 ± 346		13 ± 11	1.09 ± 0.03

Results

Table I summarizes general characteristics of 19 SGA fetuses and compares them with data of Brenner et al.⁵ Six fetuses were classified as having asymmetric IUGR on the basis of the head circumference/abdominal circumference ratio as compared with the other 13 SGA fetuses with symmetric IUGR (Fig. 1).

Fetuses with asymmetric IUGR were delivered about 5 weeks earlier than the group with symmetric IUGR (p < 0.001). Therefore fetuses with symmetric IUGR had greater birth weights than those with asymmetric IUGR (p < 0.001). All birth weights were at or below Brenner's 10th percentile. The discrepancy between actual birth weight and Brenner's 10th percentile was expressed as a percentage of the latter to estimate IUGR severity. However, the severity of IUGR did not reliably identify those fetuses that were subsequently classified with asymmetric versus symmetric growth retardation.

Gestational age was significantly correlated (second-order polynomials) with biparietal diameter, head circumference, abdominal circumference, femur length, and transverse cerebellar diameter for normally grown fetuses. Figs. 2 and 3 graphically illustrate these normal regression curves with the last ultrasonographic examination plotted for each SGA fetus before delivery. The last ultrasonographic scans for fetuses with symmetric IUGR were performed slightly later in pregnancy $(35.9 \pm 1.8 \text{ weeks})$ as compared with those of asymmetric IUGR $(33.0 \pm 3.2 \text{ weeks})$ (p < 0.02). How-

ever, all fetuses exhibited ultrasonographic evidence of IUGR before delivery. The transverse cerebellar diameter regression model qualitatively appeared to most accurately predict gestational age (50th percentile) when compared with other ultrasonographic parameters for all growth-retarded fetuses.

Table II examines SGA fetuses whose true gestational age was markedly underestimated by a particular ultrasonographic parameter on the basis of IUGR type. For all SGA cases, prediction intervals markedly underestimated gestational age on the basis of abdominal circumference (18 fetuses) or femur length (9 fetuses) alone. However, relatively fewer SGA fetuses had markedly underestimated gestational age by biparietal diameter (five fetuses), head circumference (five fetuses), and transverse cerebellar diameter (five fetuses) criteria. Biparietal diameter, head circumference, femur length, and transverse cerebellar diameter were associated with similar numbers of symmetrically growth-retarded fetuses (four to five cases in each category) with underestimated gestational age. The worse predictor, abdominal circumference, was associated with 12 symmetrically growth-retarded fetuses with markedly underestimated gestational age. An analogous but smaller pattern was also observed in six fetuses with asymmetric IUGR. Under these circumstances, six abdominal circumference and four femur length measurements were observed outside of the prediction limits. Only one asymmetrically growth-retarded fetus had underestimated gestational age on the basis of BPD or

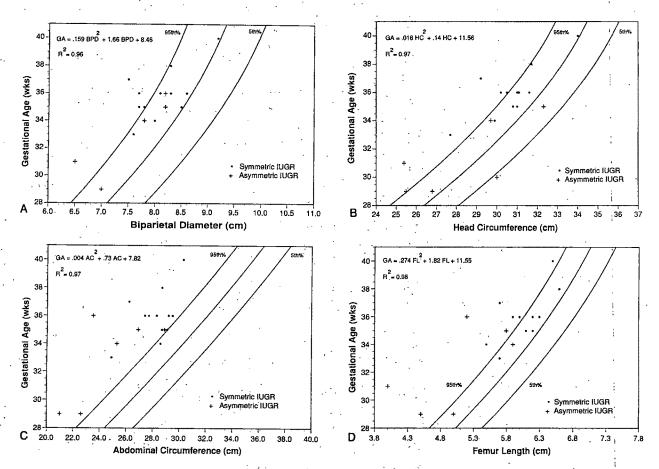


Fig. 2. Gestational age prediction intervals (95th percentile) for biparietal diameter (A), head circumference (B), abdominal circumference (C), and femur length (D) from 270 normal fetuses. Last ultrasonographic examination from 19 SGA fetuses was plotted against these curves. SGA data points are occasionally superimposed on each other. GA, Gestational age; BPD, biparietal diameter; HC, head circumference; AC; abdominal circumference; FL, femur length.

Table II. Number of SGA fetuses with underestimated gestational ages by regression models

Measurement	Asymmetric $(n = 6)$	Symmetric (n = 13)	All SGA fetuses ($N = 19$)
Biparietal diameter	1 .	4	5
Head circumference	, , <u>, , , , , , , , , , , , , , , , , </u>	4	. 5
Abdominal circumference	6	12	. 18
Femur length	4	5	9
Cerebellum diameter	0 .	5	5

Note: One fetus with asymmetric IUGR (head circumference) and one fetus with symmetric IUGR (cerebellum diameter) had measurements that overestimated gestational age.

head circumference criteria. All fetuses with asymmetric IUGR had transverse cerebellar diameter measurements that fell within the gestational age prediction limits for our normal population.

Table III outlines mean differences between observed and predicted gestational age values for multiple ultrasonographic parameters. When compared with other ultrasonographic parameters, transverse cerebellar diameter was the best overall estimator for predicting true gestational age, with a mean deviation of

about 1.3 ± 1.8 weeks from the mean regression line. Symmetric IUGR cases were associated with the smallest mean deviation from predicted gestational age, despite the fact that 38% of these fetuses actually had transverse cerebellar diameter measurements outside normal limits. Similarly, transverse cerebellar diameter and head circumference were associated with the smallest deviations from predicted gestational age for asymmetric IUGR. A statistically significant difference could not be demonstrated for the degree of gestational age

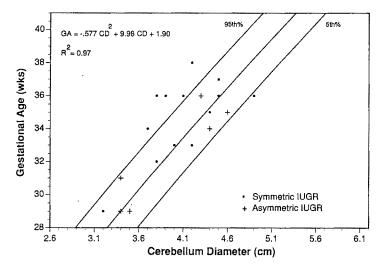


Fig. 3. Gestational age prediction intervals (95th percentile) for cerebellar diameter from 270 normal fetuses. Last ultrasonographic examination from 19 SGA fetuses was plotted against these curves. *GA*, gestational age; *CD*, transverse cerebellar diameter.

Table III. Deviation from gestational age prediction models

Measurement	Asymmetric IUGR	Symmetric IUGR	All SGA fetuses
Abdominal circumference	4.7 ± 2.3*	3.9 ± 2.0*	4.1 ± 2.1*
Femur length	$4.4 \pm 2.7*$	$3.1 \pm 1.4*$	$3.5 \pm 1.9*$
Biparietal diameter	$2.6 \pm 1.5*$	$3.9 \pm 1.6*$	$3.5 \pm 1.6*$
Head circumference	1.3 ± 2.1	$3.0 \pm 1.2*$	$2.5 \pm 1.7*$
Cerebellum diameter	0.3 ± 1.2	1.7 ± 1.9	1.3 ± 1.8

^{*}Significant difference between cerebellum diameter values at p < 0.04, unpaired t test.

deviation (transverse cerebellar diameter) observed between symmetric (1.7 \pm 1.9 weeks) versus asymmetric (0.3 \pm 1.2 weeks) IUGR (p < 0.10, NS).

Comment

The utility of transverse cerebellar diameter measurements for accurately estimating gestational age was examined in two ways. First, the quantitative deviation from predicted values (observed – expected gestational age) provided an index of how accurately the regression models predicted mean gestational age. Second, the actual number of growth-retarded fetuses whose true gestational ages would have been substantially underestimated by our regression models was summarized on the basis of prediction intervals established for our normal population.

Our findings confirm that ultrasonographic parameters such as biparietal diameter, head circumference, abdominal circumference, and femur length are likely to underestimate gestational age for growth-retarded fetuses. Head circumference measurements appear to have some advantages over biparietal diameter, femur length, and abdominal circumference on the basis of

mean differences found between observed and predicted values for asymmetric IUGR fetuses. However, two fetuses with asymmetric IUGR had head circumference measurements that actually fell outside the normal gestational age prediction limits. True gestational age appears to be more reliably predicted by transverse cerebellar diameter measurements for fetuses with asymmetric IUGR. For example, none of the fetuses with asymmetric IUGR had transverse cerebellar diameter measurements that fell outside the normal gestational age prediction curves. Furthermore, the cerebellum regression model led to the smallest differences between observed and predicted gestational age values for fetuses with asymmetric IUGR. Fetuses with symmetric growth retardation also demonstrated relative preservation of cerebellar growth but to a lesser degree than observed with asymmetric IUGR. Therefore the utility of transverse cerebellar diameter for predicting gestational age may be less applicable to a growth-retarded fetus whose head size has not been spared. This does not disprove the potential utility of transverse cerebellar diameter measurements for predicting the gestational age of fetuses with symmetric

IUGR but does suggest that caution is warranted under these circumstances.

These observations do not completely agree with previous studies that have examined the relationship of cerebellar diameter to SGA fetuses. Reece et al.3 compared 19 growth-retarded fetuses with their normal population. They were not subdivided according to IUGR type (asymmetric vs symmetric). Neonatal birth weights were ≤10th percentile according to the criteria of Usher and McLean7 or Battaglia and Lubchenco.8 All cerebellar measurements fell within normal gestational age ranges, which led to their conclusion that transverse cerebellar diameter was unaffected by growth retardation. Their findings were also similar to those recently reported by French investigators9 who also found transverse cerebellar diameter growth not to be greatly affected by 12 cases of severe IUGR. By contrast, Hill et al.4 subsequently reported severe limitations for predicting gestational age by cerebellar diameters from 44 SGA fetuses. Dating criteria was established by regular cyclic menses with an early pelvic examination before 10 weeks' gestation. The diagnosis of IUGR was confirmed by comparing birth weight with the criteria of Babson et al.10 More than half of their SGA fetuses (59%) had transverse cerebellar diameter measurements that fell >2 SD below the mean.

It is possible that studies in which transverse cerebellar diameter was found to be unaffected by growth retardation may be influenced heavily by a greater proportion of fetuses with asymmetric IUGR, because these investigators did not subclassify their subjects by head-to-body disproportion.3,9 It is presently unclear as to why our conclusions do not agree with the work reported by Hill et al., since they also subdivided their SGA fetuses on the basis of IUGR type.4 Unlike our study, they found no differences in the proportion of transverse cerebellar diameter measurements falling outside the normal range (2 SD) between symmetric (eight of 14 fetuses, 57%) versus asymmetric IUGR (18 of 30 fetuses, 60%). One major difference might have resulted from the definition of asymmetric and symmetric IUGR on the basis of head circumference/abdominal circumference ratio. Hill et al. used the original criteria of Campbell and Thoms11 to identify symmetric and asymmetric IUGR. The regression model used in our study was very similar to Campbell and Thoms' nomogram. Therefore it seems unlikely that these differences can be attributed to misclassification of symmetric versus asymmetric IUGR on the basis of head circumference/abdominal circumference ratio.

The actual influence of IUGR on the newborn primate cerebellum remains controversial. For example, Chase et al.12 studied brain weights in seven averagefor-gestational-age and four SGA infants at term gestation who died in the newborn period from a variety of causes, such as congenital heart disease, sepsis, lung disease, and subarachnoid hemorrhage. They found cerebellar weights from SGA fetuses to be reduced by 37% of appropriate-for-gestational age fetuses. Co et al.13 recently compared neonatal cerebellar dimensions by ultrasonography that involved 81 preterm infants (25 to 42 weeks) and 19 SGA infants that were matched for gestational age. Cerebellar dimensions were similar, and it was concluded that cerebellar growth was not significantly influenced by growth retardation. Unfortunately, their newborns were not classified on the basis of head-to-body disproportion. In this regard, in utero microsphere studies of term nonhuman primate fetuses (Mucaca mulatta) have also demonstrated preservation of cerebellar blood flow in spite of experimental asphyxia induced by maternal hypoxia.14 Our results support the hypothesis that human cerebellar growth is relatively resistant to IUGR when compared with other fetal structures that are commonly measured by prenatal ultrasonography.

In summary, the heterogeneous nature of IUGR will pose considerable challenges for making the diagnosis early enough to implement appropriate obstetric management. Transverse cerebellar diameter measurements appear to reasonably estimate gestational age for asymmetrically growth-retarded fetuses, where an abnormal head circumference/abdominal circumference ratio probably reflects a brain-sparing phenomenon related to uteroplacental insufficiency. Use of other common ultrasonographic measurements such as abdominal circumference and femur length may markedly underestimate gestational age under these circumstances but will provide useful information for a basis of comparison, especially when combined with the amniotic fluid status. By contrast, the ability of transverse cerebellar diameter measurements for predicting gestational age may be less applicable to fetuses with symmetric IUGR. This information should be especially useful for fetuses with suspected IUGR and poor early dating criteria when it is very difficult to distinguish between true growth retardation versus incorrect dates.

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Comparison of humerus length with femur length in fetuses with Down syndrome

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A recent report by FitzSimmons et al. demonstrated a greater frequency of upper- versus lower-extremity shortening in autopsies of second-trimester fetuses with trisomy 21. We undertook this study to determine whether this upper-limb shortening could be detected by prenatal ultrasonography in the second trimester and therefore identify fetuses at risk for trisomy 21. A restospective review of all prenatal sonograms preceding genetic amniocentesis was conducted. Between 1987 and 1990 11 consecutive fetuses between 15 and 22 weeks' gestation with trisomy 21 were identified by genetic amniocentesis. Femur and humerus lengths were plotted on growth curves created from 1470 normal patients between 12 and 26 weeks. Gestational age was confirmed by last menstrual period and biparietal diameter. In fetuses with trisomy 21, seven of 11 humeri were <5th percentile, for a sensitivity of 64%, whereas only two of 11 femurs were <5th percentile, for a sensitivity of 18%. Biparietal diameter/femur length and biparietal diameter/humerus length ratios were also tested to predict Down syndrome. In only 2 of 11 cases was the biparietal diameter/femur length ratio >95th percentile, whereas the biparietal diameter/humerus length ratio was >95th percentile in 7 of 11. Since all seven were identified by shortened humerus alone, we conclude that humerus length versus gestational age is the simplest and most effective screen. The positive predictive value of an abnormally short humerus length in detecting Down syndrome was 6.8% in our population where the prevalence of Down syndrome was 1 of 173. The present study supports the observations of FitzSimmons et al. that shortened humerus length has a greater sensitivity than femur length in cases of trisomy 21. We conclude that in fetuses at risk for trisomy 21 humerus length should be determined, because it may, if shortened, aid in the prenatal diagnosis. (AM J OBSTET GYNECOL 1991;165:1051-6.)

Key words: Down syndrome, prenatal diagnosis, humerus length

The prenatal diagnosis of Down syndrome has relied primarily on genetic amniocentesis or, more recently, on chorionic villus sampling, in women ≥35 years of age. Even if all women 35 or older underwent prenatal diagnostic testing, approximately 80% of Down syndrome would not be detected, because it occurs in the 95% of childbearing women that are under 35. Moreover, many women (even those at high risk) choose not to have invasive genetic testing for fear of miscarriage following the procedure. Therefore a noninvasive test to either detect or rule out Down syndrome would be

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beneficial. Ultrasonographically detected abnormalities associated with Down syndrome include increased nuchal skin thickening,1 short femur length, abnormal biparietal diameter/femur length ratio,2 duodenal atresia,3 omphalocele,4 cardiac defects,5 cystic hygromas,6 and nonimmune hydrops. However, these findings are not consistently found in most fetuses with Down syndrome. FitzSimmons et al.,8 using radiographs of embryo specimens, recently described long-bone growth in cases of Down syndrome and reported that shortening of the upper extremity was more pronounced than that of the lower extremity. We undertook this study to determine if the obstructions of shortened humeri of FitzSimmons et al.8 could be extended to ultrasonographic observations and thus to determine if fetal humerus length measurements in the second trimester could be helpful in detecting Down syndrome prenatally. Therefore the purpose of this study was to make second-trimester nomograms for humerus length and biparietal diameter/humerus length ratio versus gestational age and humerus length versus biparietal

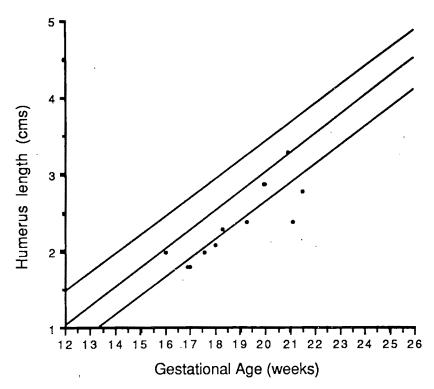


Fig. 1. Relationship between humerus length and gestational age in second trimester. Shown are 5th, 50th, and 95th percentiles. Closed circles, Fetuses with Down syndrome.

diameter in our population, to retrospectively identify fetuses with Down syndrome detected by genetic amniocentesis, to determine the sensitivity of humerus length and femur length to identify Down syndrome at different cutoff values (e.g., 5th and 10th percentiles), and to determine efficacy of an abnormal humerus length to predict Down syndrome in our population of genetic amniocentesis over a $2\frac{1}{2}$ -year period.

Material and methods

Nomograms were established from 1470 ultrasonographic examinations performed between 12 and 26 weeks' gestation in patients with a certain last menstrual period. In all cases the estimated gestational age, as established by last menstrual period, was within 1.5 weeks of the gestational age estimated by ultrasonography. Measurements obtained at each examination included biparietal diameter, head circumference, femur length, humerus length, and abdominal circumference. Fetuses with known congenital or chromosomal anomalies were excluded. This portion of the study was crosssectional; patients were included once. All examinations were performed between Jan. 1, 1988, and June 30, 1990, by members of the Division of Maternal-Fetal Medicine at the University of Connecticut Health Center and Hartford Hospital with General Electric RT 3000 (Milwaukee) and Acuson 128 (Mountain View, Calif.) real-time ultrasonography machines with freezeframe capabilities and on-screen calipers. Linear and polynomial regression analyses were performed to describe the relationships between humerus length versus gestational age, humerus length versus biparietal diameter, biparietal diameter/humerus length ratio versus gestational age, and biparietal diameter/femur length ratio versus gestational age. The 5th, 10th, 50th, 90th, and 95th percentiles were calculated. All patients who come through our antepartum testing unit are given outcome cards to complete; about 75% return these.

Records of all patients undergoing second-trimester genetic amniocenteses over the 2½-year study period were reviewed to identify all cases of Down syndrome diagnosed prenatally and to determine the prevalence of Down syndrome in this population. Over this study period, 1907 genetic amniocenteses were performed. Indications included advanced maternal age (77%), low maternal serum α-fetoprotein value (20%), and previous child with trisomy (3%). At the time of amniocentesis, ultrasonographic measurements including biparietal diameter, head circumference, femur length, humerus length, and abdominal circumference were obtained.

Results

During the study period 11 fetuses with Down syndrome were identified by second trimester genetic am-

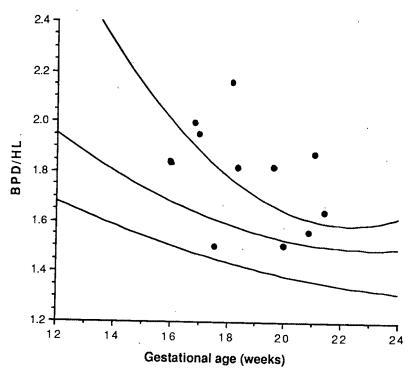


Fig. 2. Relationship between biparietal diameter/humerus length (BPD/HL) ratio versus gestational age. Shown are 5th, 50th, and 95th percentiles. Closed circles, Fetuses with Down syndrome.

niocentesis. We compared the humerus length in these 11 cases with the curves established in the 1470 normal patients (Fig. 1) and found that in seven of 11 cases (sensitivity, 64%) the humerus length fell below the 5th percentile from the norm for that gestational age. If the 10th percentile was chosen as a cutoff, the sensitivity remained at 64%. In all 11 cases, the humerus length fell below the 50th percentile. The relationship between humerus length and gestational age is described by the linear equation humerus length = 0.241 gestational age -1.787 (r = 0.939, $R^2 = 0.88$, p = 0.0001). The biparietal diameter/humerus length ratio versus gestational age is illustrated in Fig. 2; the relationship is described by the second-order polynomial equation biparietal diameter/humerus length = 3.834 - 0.204 gestational age + 0.004 gestational age² (r = 0.517, $R^2 = 0.26, p = 0.0001$). Seven of 11 fetuses with Down syndrome had biparietal diameter/humerus length ratios >95th percentile, with the same seven being <90th percentile with no new cases identified. The relationship between humerus length and biparietal diameter is shown in Fig. 3 and is described by the linear equation humerus length = 1.146 biparietal diameter + 1.14 $(r = 0.936, R^2 = 0.877, p = 0.0001)$. Five of 11 cases (45%) of Down syndrome had humerus length <5th percentile, with the sensitivity improving to seven of 11 (64%) if the 10th percentile was used as the cutoff. During the study period, six cases of trisomy 18 and no cases of trisomy 13 were identified. Only two of the six cases (sensitivity, 33%) of trisomy 18 had humerus length <5th percentile.

Abnormal femur length measurements were not as sensitive in detecting Down syndrome in comparison with humerus length. When femur length versus gestational age was considered, only two of 11 cases were below the 5th percentile and three of 11 below the 10th percentile. The biparietal diameter/femur length ratio versus gestational age (Fig. 4) identified two of 11 cases and three of 11 cases at the 95th and 90th percentile, respectively. The relationship between biparietal diameter/femur length and gestational age is described by the second-order polynomial equation biparietal diameter/femur length = 3.82 - 0.197 gestational age + 0.004 gestational age² (r = 0.59, $R^2 = 0.348$, p = 0.0001). Femur length versus gestational age identified only one of 11 and three of 11 cases at the 95th and 90th percentiles, respectively.

We concluded from these analyses that the humerus length versus gestational age was the simplest and most efficacious and thus the most practical ultrasonographic screening test for Down syndrome. The 5th percentile humerus length for gestational ages from 12 to 26 weeks is shown in Table I. Over the 21/2-year study period, 1907 genetic amniocenteses were performed at the University of Connecticut Health Center (1431 cases) and Hartford Hospital (476 cases). Eleven cases of Down syndrome were identified in the 1907 cases, for an overall prevalence of 1 in 173. The sensitivity,

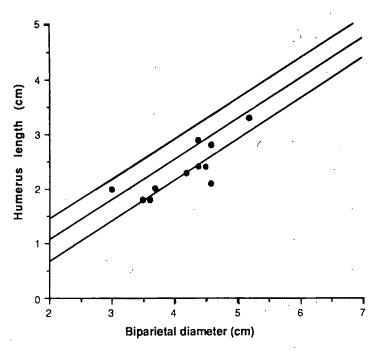


Fig. 3. Relationship between humerus length and biparietal diameter throughout second trimester. *Closed circles*, Fetuses with Down syndrome.

Table I. Cutoff values (5th percentile) for humerus length from 12 to 26 weeks' gestation.

Gestational age (completed weeks)	Abnormally short (5th percentile) humerus length (mm)	
. 12	7	
13	9	
14	12	
15	14	
16	16	
. 17	19	
18	ÌΙ	
19	24	
20	26	
21	29	
. 22	31	
23	34	
24	36	
25	. 38	
26	41	

specificity, and positive and negative predictive value of humerus length versus gestational age is shown in Table II. An abnormally short femur length was not as sensitive as humerus length in detecting Down syndrome, as indicated in Table III. The positive predictive value of an abnormally short (<5th percentile) humerus length was 6.8% in our population of patients seen for genetic amniocentesis, who had a prevalence of Down syndrome of 1 in 173. Moreover, a normal humerus length measurement (≥5th percentile) would rule out Down syndrome in 99.7% of cases (negative predictive value).

Comment

Since 1970, very little has changed in regard to prenatal diagnosis of Down syndrome. The emphasis has been on testing women of advanced maternal age, although only 20% of Down syndrome occurs in that age group. Recently, maternal serum α -fetoprotein testing has been incorporated into the prenatal diagnostic testing schema, because women who are carrying fetuses with Down syndrome have been shown to have lower levels of maternal serum α -fetoprotein. If all women under 35 had routine maternal serum α-fetoprotein screening, another 20% of Down syndrome cases would be identified.9 More recently, fetal ultrasonography has been suggested as an additional tool to aid in prenatal detection of Down syndrome. However, this method has not been very practical for the average clinician for several reasons. First, while some anomalies are frequently associated with Down syndrome they are not present in the majority of cases. For example, while 30% to 40% of fetuses with duodenal atresia prove to have Down syndrome, only 5% of fetuses with Down syndrome have duodenal atresia. 10 Second, some of the anomalies associated with Down syndrome are not readily detectable until the third trimester, when elective pregnancy termination is no longer an option for the parents. Duodenal atresia is one example, where the classic "double bubble" finding in association with polyhydramnios is not usually visualized before 24 weeks.3 Third, some of the ultrasonographic findings may not be readily apparent or easy to obtain for the average sonographer on a routine examination.

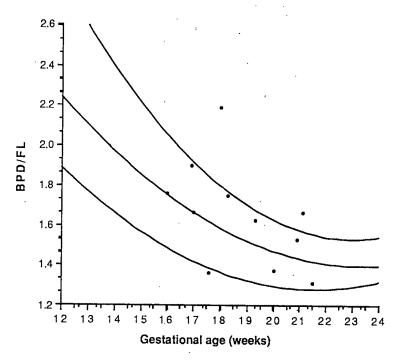


Fig. 4. Relationship between biparietal diameter/femur length (BPD/FL) ratio versus gestational age. Shown are 5th, 50th, and 90th percentiles. Closed circles, Fetuses with Down syndrome.

Examples include nuchal skin thickening, cardiac defects, and hypoplasia of the middle phalanx of the fifth finger.11 Fourth, some mathematic calculations may be required, as in the case of biparietal diameter/femur length ratio. Moreover, the sensitivity of this ratio to detect Down syndrome has recently been questioned.12

Humerus length measurements, on the other hand, are easy to obtain. Long-bone measurements, particularly the femur length, are included in most, if not all, second-trimester ultrasonographic examinations. Including the humerus length as part of the routine examination requires no special expertise in obstetric ultrasonography and would add very little time to the examination. No ratios need to be calculated. In our population 64% of cases of Down syndrome would have been identified by this simple prenatal screening method, whereas <5% of normal fetuses had an abnormally short humerus length.

Our findings appear to agree with the recent observations of Benacerraf et al.18 They found that if the actual-to-expected humerus length ratio was ≤0.90, they were able to detect 12 of 24 fetuses with Down syndrome (sensitivity, 50%) with a 6.25% false-positive

It would appear that if our findings are confirmed, fetal humerus length measurements should be obtained at any second-trimester ultrasonographic examination. If the humerus length falls below the 5th percentile, prenatal genetic testing should be offered to the pa-

Table II. Efficacy of humerus length to predict Down syndrome

	Down syndrome	Normal	Total
Humerus length <5th percentile	7	95	102
Humerus length ≥5th percentile	4	1795	1799
TOTAL	11	1890	1901*

Sensitivity, 7 of 11 (64%); specificity, 1795 of 1890 (95%); positive predictive value, 7 of 102 (7%); negative predictive value, 1795 of 1799 (99.7%).

Table III. Efficacy of femur length to predict Down syndrome

	Down syndrome	Normal	Total
Femur length <5th percentile	2	95	97
Femur length ≥5th percentile	9	1795	1804
TOTAL	11	1890	1901*

Sensitivity; 2 of 11 (18%); specificity; 1795 of 1890 (95%); positive predictive value; 2 of 97 (2%); negative predictive value; 1795 of 1804 (99.5%).

^{*}The six cases of trisomy 18 were excluded.

^{*}The six cases of trisomy 18 were excluded.

tient. This may prove to be an effective way of identifying fetuses with Down syndrome in a low-risk population, specifically women <35 years old. We are prospectively evaluating the efficacy of an abnormal humerus length to predict Down syndrome in such low-risk women.

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Ultrasonographic diagnosis of congenital anomalies in twins

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To determine whether serial ultrasonographic examinations with basic anatomic surveys provide an adequate screen for congenital abnormalities that are more common in twins, we compared the results of prenatal sonograms and neonatal examinations for 314 twins (157 pairs) delivered during a recent 42-month period. An anomaly was defined as major if it potentially required surgical repair or precluded normal life expectancy; otherwise it was defined as minor. Thirty-three twins (9.5%) had 40 anomalies; 28 (9%) were major and 12 (4%) were minor. Prenatal ultrasonography with cardiac screening limited to the four-chamber view provided detection of 39% of all major anomalies, 55% of noncardiac major anomalies but none of the cardiac lesions, and 69% of the major anomalies for which routine prenatal management should be altered. No false-positive diagnoses incorrectly altered management. We conclude that serial prenatal ultrasonographic examinations are useful in detecting noncardiac anomalies for which twins are at increased risk, but the four-chamber view is not an adequate screen for the cardiac malformations of twins. (Am J Obstet Gynecol 1991;165:1056-60.)

Key words: Prenatal diagnosis, twins, ultrasonographic, congenital malformations

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Twins are known to have an increased risk of congenital anomalies, up to threefold higher than for singletons¹⁻⁵; however, the value of ultrasonography in the diagnosis and management of the anomalies of twins has not been proved. To determine whether ultrasonographic examinations that include a basic ana-

tomic survey provide an adequate screen for clinically significant congenital defects in twins, we compared the results of prenatal sonograms and neonatal examinations for twins delivered during a recent 42-month period.

Material and methods

Subjects. Mothers whose ultrasonographic examinations were included in the study received their prenatal care from the Department of Obstetrics and Gynecology of the University of Florida and were delivered at Shands Hospital. The twins were born between Sept. 1, 1986, and Jan. 31, 1990. Details of the sonograms were obtained from each mother's prenatal record. Additional perinatal data were obtained from a computerized data base and the registry of the labor and delivery unit.

The University of Florida Department of Obstetrics and Gynecology provides prenatal care and ultrasonographic services for high-risk patients referred from the county health clinics of north-central Florida. Fiftyone percent of the generally low-income patients are white, 47% are black, and 2% are Hispanic or Asian.

Methods. Each twin pair had at least one prenatal sonogram available for review. Ultrasonographic examinations were scheduled every 3 to 4 weeks and were performed according to the recommendations of the American College of Obstetricians and Gynecologists, including evaluation of fetal number and presentation, amniotic fluid volume, gestational dating, placental localization, and an anatomic survey of the head, heart (four-chamber view), spine, stomach, kidneys, bladder, abdominal wall, and extremities.6,7 Each examination was performed by a registered diagnostic medical sonographer or a perinatologist. A congenital anomaly was defined as major if it potentially required surgical repair or precluded normal life expectancy; otherwise it was defined as minor.8

To determine the accuracy of ultrasonography in determining congenital abnormalities, ultrasonographic findings were compared with those of physical examinations performed by staff neonatologists. Fetal gender and membrane chorionicity and amnionicity were reviewed to potentially determine the zygosity of twin pairs in pregnancies complicated by congenital malformations, but other tests to confirm zygosity were not performed. Because abnormalities were relatively infrequent, only descriptive statistics are presented.

Results

During the study period, 342 twins (171 pairs) were delivered, for an incidence of twin gestations of 1.6%. Of these, 314 (157 twin pairs) had at least one prenatal ultrasonographic examination and were included in the study population. The mean number of sonograms per

patient was 2.5. Thirty-three infants (9.5%) had 40 anomalies detected during neonatal examination, including two twins with multiple major malformations. Of these 40 malformations, 28 (9%) were major and 12 (4%) were minor.

The most common major anomalies involved the genitourinary system, with an incidence of 14 in 314 (5%), as indicated in Table I. Six (43%) of these were detected by prenatal ultrasonographic examination. With serial examinations, one case of hydronephrosis was detected on a third-trimester scan after a normal scan during the second trimester. The next most common major abnormalities were cardiovascular, with eight structural defects documented in five infants. None of these lesions were diagnosed prenatally by ultrasonography. However, prenatal ultrasonographic examinations did detect all cases of nonimmune hydrops, osteogenesis imperfecta (IB), and omphalocele. None of the minor anomalies was detected prenatally. No false-positive prenatal diagnoses were made.

Serial basic ultrasonographic examinations provided 39% sensitivity, 100% specificity, 100% positive predictive value, and 91% negative predictive value for the detection of all major congenital anomalies; however, prenatal diagnosis by ultrasonography would not be expected for several of these anomalies. Table II shows the sensitivity of prenatal ultrasonography in this population for the diagnosis of those anomalies reportedly predictable by ultrasonography.

Major anomalies in this twin population that might alter routine prenatal management are hydronephrosis, bladder outlet obstruction, bilateral renal dysplasia, cloacal exstrophy, tricuspid atresia, pulmonary stenosis, right ventricular hypoplasia, nonimmune hydrops, osteogenesis imperfecta (IB), and omphalocele. Of 16 such malformations, 11 were correctly diagnosed by routine prenatal ultrasonography, yielding a sensitivity of 69%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 95%.

Among the 33 twins with congenital malformations, 5 (15%) were monochorionic and thus certainly monozygotic; 16 (49%) were dichorionic, of the opposite sex and therefore dizygotic; and 12 (36%) were dichorionic, of the same sex and therefore of unsure zygosity. Five twin pairs had concordant malformations. One dizygotic pair had osteogenesis imperfecta (IB) and one monozygotic pair had hydroceles. Two pairs of unsure zygosity (dichorionic, same sex) were concordant for hypospadias and hydroceles, respectively. Another twin pair of unsure zygosity, concordant for tricuspid atresia, had other dissimilar major anomalies.

Comment

When performed in accordance with the recommendations of the American College of Obstetricians and

Table I. Incidence and ultrasonographic diagnosis of congenital malformations in twins

,	No. diagnosed prenatally/No. diagnosed neonatally		
	Individual malformations	Malformations by system	Malformations major or minor
Major congenital malformations			
Ğenitourinary system	• •		
Hypospadias	0/5		
Hydronephrosis	2/3*		
Bladder outlet obstruction	2/2	•	
Bilateral renal dysplasia	1/1		
Inguinal hernia	0/1		k .
Cloacal exstrophy	1/1†		
Unilateral renal agenesis	0/1†		
SUBTOTAL		6/14	
Cardiovascular system			
Ventricular septal defect	0/3±		
Tricuspid atresia	0/2\$		
Pulmonary stenosis	0/1†		
Right ventricular hypoplasia	0/1†		
Patent ductus arteriosus	0/1		
SUBTOTAL		0/8	
Miscellaneous			
Imperforate anus	0/1†		
Nonimmune hydrops .	1/1	* *	
Osteogenesis imperfecta (IB)	2/2 "		•
Omphalocele	· 2/2†		
SUBTOTAL		5/6	
TOTAL		_	11/28
Minor congenital malformations			
Polydactyly	0/3	•	
Hydrocele.	0/8		
Cavernous hemangioma	0/1		
TOTAL			0/12

^{*}One of three detected at third-trimester scan after normal second-trimester scan.

Gynecologists, serial prenatal ultrasonographic examinations, which included a basic anatomic survey, were predictive of the majority of noncardiac major congenital malformations in twins. Considering only those malformations for which a prenatal diagnosis would lead to an alteration in routine perinatal management, serial basic ultrasonographic evaluations had a sensitivity of 69%, allowing appropriate planning of fetal surveillance and timing and method of delivery for most patients. No false-positive diagnoses led to inappropriate perinatal management.

The low overall sensitivity of prenatal ultrasonographic examinations in this study may be attributed to poor detection of cardiovascular anomalies, the second most common group of malformations. As are other anomalies, cardiovascular malformations are as much as twice as common among twins as among singletons. Since these cardiac structural defects are frequently of great physiologic significance, adequate screening for them is important. Although detailed

echocardiography has been reported as beneficial for some groups specifically at increased risks for fetal cardiac anomalies,9 a basic ultrasonographic examination with a four-chamber cardiac view, as performed in this study, generally has been regarded as an adequate screen. Copel et al.10 reported a 92% sensitivity for the detection of cardiac structural defects using the fourchamber view as the primary screening technique. They attributed the imperfect sensitivity to difficulties in detecting uncomplicated ventricular septal defect, semilunar valve stenosis, coarctation of the aorta, and simple transposition. Of those lesions potentially missed with a four-chamber view, only simple ventricular septal defect is specifically noted to be more common in twins.5 A false-negative four-chamber cardiac ultrasonographic examination for simple ventricular septal defect will not alter perinatal outcome, but missing a malformation of greater physiologic significance, such as pulmonary stenosis, may indeed have a negative impact on perinatal outcome. Given the increased risk of

[†]Second twin with multiple major malformations.

[‡]First twin with multiple major malformations.

[§]Both twins with multiple major malformations.

^{||}Nonimmune hydrops in association with twin-twin transfusion.

Table II. Sensitivity of ultrasonographic diagnosis of major congenital malformations in twins

Malformations		Sensitivity*	
Genitourinary system	•	1	
Hydronephrosis	2/3 (67%)		
Bladder outlet obstruction	2/2 (100%)		
Bilateral renal dysplasia	1/1 (100%)		
Cloacal exstrophy	1/1 (100%)		
Unilateral renal agenesis	0/1 (0%)		
SUBTOTAL .	, ,	6/8	(75%)
Cardiovascular system			, ,
Ventricular septal defect	0/3 (0%)		
Tricuspid atresia	0/2 (0%)		
Pulmonary stenosis	0/1 (0%)		
Right ventricular hypoplasia	0/1 (0%)		
SUBTOTAL	, ,	0/7	(0%)
Miscellaneous			
Nonimmune hydrops fetalis	1/1 (100%)		
Osteogenesis imperfecta	2/2 (100%)		
Omphalocele	2/2 (100%)		
SUBTOTAL		5/5	(100%)
TOTAL		11/20	(55%)

^{*}Number diagnosed prenatally per number diagnosable prenatally.

cardiac structural defects in twins and the insensitivity of the routine four-chamber cardiac view for some of the malformations, echocardiographic evaluation of the aortic and pulmonary outflow tracts, the arch of the aorta, and ventricular size could increase the sensitivity of the screening protocol.

The genitourinary system demonstrated the highest incidence of structural anomalies among twins in this study, yet prenatal diagnosis would be neither expected nor helpful in perinatal management in nearly half of these abnormalities (hypospadias and inguinal hernia). Prenatal ultrasonography did detect 86% (6/7) of the genitourinary lesions for which perinatal management might be altered.

The increased risk for congenital anomalies among twins has been reported as limited to those of the same sex3.4 and, more specifically, monozygotic twins.5 Single members of five monozygotic twin pairs were affected by congenital anomalies, including nonimmune hydrops (in association with twin-twin transfusion), hydronephrosis, hypospadias, and hydrocele. An undefined subset of 12 dichorionic same-sex twin pairs with at least one malformed member also may have been monozygotic. On comparison of the proportion of monozygotic congenitally malformed twins in this study (≥15%) with the similar proportion of monozygotic twins in the general twin population (approximately 20%), we cannot confirm an increased incidence of congenital malformations in monozygotic twins. Concordance for abnormalities also has been reported as higher among same-sex twins3 and monozygotic twins.5 Six of the 28 (21%) major congenital anomalies occurred concordantly in three twin pairs, including one quarter (4/16) of those major anomalies for which prenatal care may be altered. This suggests that the identification of a major congenital anomaly in one twin of a same-sex set should prompt a careful search, particularly for a related anomaly, in the other twin.

Although recommended for the sequential assessment of growth, serial anatomic surveys also proved useful for prenatal diagnosis of malformations in this study. After a prior normal second-trimester sonogram, hydronephrosis was first detected in one fetus during a third-trimester scan.

Even a single occurrence of some of the abnormalities included in a study of this size yields an incidence greater than that reported elsewhere for either single or multiple gestations. The limited sample size also may explain the absence of some congenital abnormalities reported to be more common among twins, such as neural tube defects and facial clefts.5 Limitations attributable to the sample size of this study are recognized; the purpose of our study is to assess the accuracy of ultrasonographic prenatal diagnosis in twins, not to define the scope of malformations in this population.

We conclude that serial prenatal ultrasonographic examinations that include a basic anatomic survey are useful in detecting the noncardiac anomalies for which twins are at increased risk but that the four-chamber cardiac view in not an adequate screen for cardiac structural malformations of twins.

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Does amniotic fluid index affect the accuracy of estimated fetal weight in preterm premature rupture of membranes?

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Estimated fetal weights play a critical role in the management scheme of patients with preterm premature rupture of membranes but are often technically difficult to obtain in these patients because of low amniotic fluid volume. Previous studies have had conflicting data as to the accuracy of estimated fetal weights in preterm premature rupture of membranes. This study was undertaken to evaluate the effect of amniotic fluid index on the accuracy of estimated fetal weights in pregnancies complicated by preterm premature rupture of membranes. Over a 2-year period at Long Beach Memorial Medical Center, 98 patients with preterm premature rupture of membranes who had an ultrasonographic examination with estimated fetal weights and amniotic fluid index performed within 48 hours of delivery were identified and compared with a control group of 55 patients in preterm labor with normal amniotic fluid index for gestational age, also obtained within 48 hours of delivery. Shepard and Hadlock formulas were used to estimate fetal weight. Results were measured in percent error from the actual birth weight. All birth weights were <2000 gm. No statistical differences were identified. The value of amniotic fluid index did not affect the accuracy of predicted estimated fetal weight in preterm premature rupture of membranes. Predicted estimated fetal weight of patients with preterm premature rupture of membranes appears to be as accurate as predicted estimated fetal weight in pregnancies with normal amniotic fluid volumes. (AM J OBSTET GYNECOL. 1991;165:1060-2.)

Key words: Estimated fetal weight, preterm premature rupture of membranes, amniotic fluid index

Ultrasonographically estimated fetal weight is often used to make critical management decisions in preterm

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premature rupture of membranes. Previous studies have had conflicting reports regarding the reliability of estimated fetal weight, leaving the obstetrician unsure of the usefulness of these data. It has been suggested that low amniotic fluid volume leads to compression of the head and abdomen, in addition to reducing the resolution of the ultrasonographic image, rendering the estimated fetal weight inaccurate and unreliable.^{1,2}

No previous study has evaluated the effect of accuracy of estimated fetal weights and how this correlated

with amniotic fluid index in pregnancies complicated by preterm premature rupture of membranes. This study was designed to evaluate the effect of oligohydramnios on the accuracy of estimated fetal weights in pregnancies complicated by preterm premature rupture of membranes.

Material and methods

Medical records of 98 patients delivered at Long Beach Memorial Women's Medical Center with the diagnosis of preterm premature rupture of membranes and birth weights of <2000 gm were retrospectively analyzed. Preterm premature rupture of membranes was defined as rupture of membranes occurring before 36 weeks. It was confirmed in all patients by sterile speculum examination with pooled fluid, ferning, and alkaline pH determinations (Nitrazine test). All ultrasonographic measurements and evaluations were performed by the perinatal staff within 48 hours of delivery.

Amniotic fluid indexes were determined by the fourquadrant technique, as described by Phelan et al.3 Patients were stratified into four groups according to the degree of oligohydramnios detected. Estimated fetal weights were calculated by using Shepard et al.4 (biparietal diameter, abdominal circumference) and Hadlock et al.5 (femur length, abdominal circumference) formulas.

The control group, matched for gestational age and birth weight, consisted of 55 patients in preterm labor with intact membranes and normal amniotic fluid index for gestational age. Ultrasonographic examinations also were performed in the control group within 48 hours of delivery and by the same perinatal staff.

Power analysis predicted an 80% chance of detecting a 30% difference in the percent error from actual birth weight in a total population of 98 patients. Data were analyzed with the Wilcoxon rank sum test with a p value < 0.05 considered statistically significant. All comparisons were against a two-sided alternative hypothesis expressed as percent error ± 1 SD from actual birth weight.

Results

Table I depicts the effect of amniotic fluid index measurements on the accuracy of predicted estimated fetal weight assessed as percent error of estimated fetal weight from actual birth weights, with the Shepard formula. Table II describes the same relationship with the Hadlock formula. The patients were grouped with regard to degree of oligohydramnios, with 43 noted to have an amniotic fluid index between 0.0 and 5.0. Not depicted in the table are 19 of these 43 patients who were determined to have severe oligohydramnios with an amniotic fluid index between 0.0 and 2.0. Twentynine patients had an amniotic fluid index between 5.9

Table I. Estimated fetal weight with the Shepard formula

Amniotic fluid index (cm)	No.	Estimated , fetal weight*	
0.0-5.0	43	9.42 ± 7.2	
5.1 - 9.9	29	8.57 ± 6.7	
>10	26	10.12 ± 8.5	
Control	55	9.65 ± 8.5	

^{*}Percent error ± SD.

Table II. Estimated fetal weight with the Hadlock formula

F	Amniotic fluid index (cm)	No.	Estimated fetal weight*	
	0.0-5.0	43	9.15 ± 7.2	
	5.1-9.9	29	9.87 ± 6.9	
	>10	26	12.18 ± 10.7	
	Control	55	10.88 ± 8.8	

^{*}Percent error ± SD.

Table III. Correlation of birth weight and estimated fetal weight in preterm premature rupture of membranes

Birth weight (gm)	No.	Shepard*	Hadlock*
500-1000	35	9.65 ± 8.5	11.15 ± 9.9
1001-1500	27	9.27 ± 6.7	10.74 ± 8.2
1501-2000	36	9.40 ± 6.6	9.47 ± 6.6

^{*}Percent error ± SD.

and 9.9, and 26 patients were determined to have fluid indexes of >10. No statistical difference was noted in percent error between the study patients with preterm premature rupture of membranes and the control group with intact membranes, or between the subgroups of amniotic fluid volume. In addition, there were no statistical differences in the percent error calculated by either formula.

Patients also were stratified with regard to birth weight, with 35 infants weighing between 500 and 1000 gm. As depicted in Table III, both formulas appear to be equivalent in their ability to predict estimated fetal weight with varying birth weights, even when addressing the very-low-birth-weight groups.

Comment

Estimation of fetal weight plays a critical role in the decision-making and management scheme of patients with preterm premature rupture of membranes. However, the literature addressing the issue of accuracy of estimated fetal weight in the face of oligohydramnios remains confusing and sparse. All the studies examining estimated fetal weight in patients with preterm premature rupture of membranes published to date lack a concise and quantifiable definition of oligohydramnios and "low fluid volume," rendering the data difficult to interpret.

In 1985, O'Keefe et al.¹ were the first authors to report that biparietal diameter measurements in patients with preterm premature rupture of membranes were inaccurate in 45% of their 100 patients as assessed by an abnormal cephalic index and concluded that biparietal diameter measurements were unreliable in estimating fetal weight in patients with preterm premature rupture of membranes.

This study did not quantify or justify the severity of oligohydramnios that resulted in distortion of the contour of the head. In 1986 a similar study looking at the effects of oligohydramnios on estimation of fetal weight by Ott et al.2 concluded that the cephalic index was not appreciably distorted by oligohydramnios. Again, that study did not define the degree of oligohydramnios observed in study patients. Subsequently, Bottoms et al.6 reported an estimation of fetal weight in 26 pregnancies complicated by premature rupture of the membranes. Although all measurements were smaller in patients with premature rupture of the membrane than in control patients, the authors attributed this finding to compromised intrauterine growth rather than artifacts from physical compression caused by oligohydramnios.

Benacerraf et al.⁷ in 1988 looked at the accuracy of ultrasonographically estimated fetal weight in a large series of 1301 patients with varying amounts of amniotic fluid volume and noted that the presence of oligohydramnios made no difference in the percent errors. In this study 17% were noted subjectively to have oligohydramnios and only 20 of 1301 fetuses had birth weights <2000 gm.

Townsend et al.⁸ in 1988 looked specifically at ultrasonographically estimated fetal weight in 53 very-low-birth-weight infants (<1000 gm). Twenty-four fetuses were noted to have "decreased" amniotic fluid volume subjectively. The authors reported that no statistical differences in accuracy of weight prediction in preterm deliveries were observed when patients with normal amniotic fluid were compared with those with oligohydramnios. Finally, in 1990 Valea et al.⁹ compared 86 patients with preterm premature rupture of membranes at ≤36 weeks with 112 control patients with intact membranes matched for gestational age and were able to demonstrate that amniotic fluid volume was not an important variable in the accuracy of ultrasono-

graphically estimated fetal weights. However, in this study there was no mention of the percentage of patients with oligohydramnios, and the amniotic fluid was not quantified.

In our study we quantified the degree of oligohydramnios and demonstrated in support of the cited studies that low amniotic volumes do not appreciably affect the accuracy of ultrasonographically estimated fetal weights in patients with preterm premature rupture of membranes. Nineteen of our study patients had severe oligohydramnios with an amniotic fluid index of 0 to 2 cm. In spite of this severe lack of fluid, the percent error from actual birth weight was only 7.45 ± 7.2 by the Shepard formula and 9.15 ± 7.2 by the Hadlock formula. Predicted estimated fetal weights in preterm gestations with preterm premature rupture of membranes appear to be as accurate as those predicted in patients with normal amniotic fluid volume, even in very-low-birth-weight infants.

We conclude that, in the hands of experienced ultrasonographers, predicted estimated fetal weights may be used with confidence in the management of pregnant patients with oligohydramnios.

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Transcervical chorionic villus sampling and midtrimester oligohydramnios

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To study the possible association between transcervical chorionic villus sampling and midtrimester oligohydramnios, we conducted a prospective cohort study of all women who were seen for genetic counseling in the first trimester during a 2-year period. Women who chose chorionic villus sampling were compared with women who chose traditional amniocentesis for incidence of midtrimester oligohydramnios. Of 442 women exposed to chorionic villus sampling with a normal fetal karyotype, severe oligonydramnios developed in 12 (2.7%) at 16 to 23 weeks' gestation. None of the 391 women with normal fetal karyotypes who were counseled at the same time in pregnancy but who chose amniocentesis had oligohydramnios at the time of amniocentesis (p = 0.01). A nested case-control analysis was performed within the chorionic villus sampling group to evaluate risk factors associated with midtrimester oligohydramnios. Midtrimester oligohydramnios occurring after chorionic villus sampling was associated with postprocedure bleeding and elevated maternal serum α -fetoprotein (p < 0.01). There were no perinatal survivors with midtrimester oligohydramnios. (Am J OBSTET GYNECOL 1991;165:1063-8.)

Key words: Chorionic villus sampling, amniocentesis, oligohydramnios

First-trimester transcervical chorionic villus sampling is now widely available for early prenatal diagnosis of chromosomal and metabolic abnormalities. Pregnancy loss after this procedure is reported to be 2% to 5%.1-6 The pregnancy loss rate after midtrimester amniocentesis is reported to be 0.5% to 1.0%.7-8

It has been suggested that midtrimester oligohydramnios may be a late complication of chorionic villus sampling,3,9 although this assertion has not been systematically evaluated. In fact, the exact frequency of midtrimester oligohydramnios remains uncertain. Several studies 10-12 have addressed the poor prognosis of midtrimester oligohydramnios and its association with fetal abnormalities, but descriptive data on the prevalence of midtrimester oligohydramnios in the population are generally lacking.

To study the possible association between transcervical chorionic villus sampling and midtrimester oligohydramnios, we conducted a prospective cohort

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seling in the first trimester during a 2-year period. Women selecting chorionic villus sampling were compared with those women selecting traditional amniocentesis for the occurrence of midtrimester oligohydramnios. A separate cohort of women not seen until the midtrimester for amniocentesis were studied during the same time period to estimate a population prevalence of midtrimester oligohydramnios.

study of all women who were seen for genetic coun-

Material and methods

The study population consisted of all women referred for genetic counseling during the first trimester at Swedish Hospital Medical Center, Seattle, Washington, between Jan. 1, 1988, and Dec. 31, 1989, inclusive.

Patients were seen at 6 to 11 weeks from the last menstrual period and underwent an ultrasonographic examination on the initial visit for confirmation of fetal viability and for ascertainment of gestational age. Patients also received counseling regarding chorionic villus sampling and amniocentesis, with the choice of procedure left to the patient. Patients electing chorionic villus sampling were scheduled for the procedure at 9 to 12 weeks (menstrual), and those patients electing amniocentesis (amniocentesis group A) were scheduled for the procedure at 15 to 20 weeks.

Exclusion criteria for chorionic villus sampling included active vaginal infection (herpes, streptococcus, yeast), twins, severely retroverted uterus, or vaginal bleeding—either clinically within 1 week of procedure or as a perigestational hemorrhage seen on ultrasonography at the time of scheduled procedure. These patients were scheduled for amniocentesis at 15 to 20 weeks.

Before the chorionic villus sampling procedure, an ultrasonographic examination was performed to determine uterine position, placental location, and gestational age. After sterile cervical preparation, chorionic villus sampling was performed by one of two obstetricians using a 5.7 Fr Cook chorionic villus sampling catheter under continuous ultrasonographic observation. Up to three separate attempts were performed (separate catheter with each attempt) to obtain adequate villi (approximately 10 mg). The procedure was terminated as soon as adequate villi were obtained. Patients were seen at 24 to 48 hours after procedure for confirmation of fetal viability by ultrasonography and for assessment of amniotic fluid volume and interval complications, such as bleeding, uterine cramping, leakage of amniotic fluid, or fever. At 15 to 20 weeks patients had a maternal serum α-fetoprotein (AFP) assay and a complete ultrasonographic examination to assess interval growth, amniotic fluid volume, and fetal anomalies. Pregnancy outcome data were obtained on all patients.

Patients electing amniocentesis (amniocentesis group A) were scheduled for the procedure at 15 to 20 menstrual weeks. Before the procedure, a complete ultrasonographic examination was repeated to assess interval growth (from 6 to 11 weeks before counseling ultrasonography), placental location, amniotic fluid volume, and fetal anomalies. Amniocentesis was performed by one of four obstetricians, using a 22-gauge spinal needle under continuous ultrasonographic guidance. Twenty-five milliliters of amniotic fluid was withdrawn for fetal karyotype and amniotic fluid AFP assay. Pregnancy outcome data were obtained from existing follow-up protocols in place since 1973, which call for telephone and letter contact by both referring physician and patient for any untoward pregnancy outcome after amniocentesis. This group served as the control group to compare risks of midtrimester oligohydramnios with the chorionic villus sampling group.

A separate cohort of women, amniocentesis group B, was used to assess the baseline prevalence of oligohydramnios in the midtrimester. This cohort consisted of all women seen during the study period for amniocentesis in the midtrimester because of advanced maternal age or prior fetal chromosome anomaly only. Multiple gestation and high and low maternal serum AFP cases were excluded. The protocol followed with this group was identical to that followed by the amniocentesis group A.

The genetic counseling and hospital medical records of all study participants were reviewed for information pertaining to the procedure chosen, procedural complications and results, pregnancy outcome, and demographic characteristics. Cases with abnormal karyotypes and recognizable fetal malformations were excluded from further analysis. For the purposes of this study, oligohydramnios was defined as decreased amniotic fluid observed on ultrasonography with the deepest pocket of amniotic fluid being ≤1 cm in depth.

Initial comparisons on the occurrence of midtrimester oligohydramnios were made with Fisher's exact test between midtrimester ultrasonographic examinations of women exposed to first-trimester chorionic villus sampling and women undergoing first-trimester ultrasonography and counseling for chorionic villus sampling but who elected midtrimester amniocentesis (amniocentesis group A).

A nested case-control analysis was conducted on all women who elected to have chorionic villus sampling. Cases of oligohydramnios detected between 16 and 20 weeks' gestation were compared with controls with normal amniotic fluid volume levels during the same time period. Comparisons between cases and controls were made with Fisher's exact and t tests for maternal characteristics, amount of villi sampled, number of catheter insertions, operator, procedural complications, chorionic villus sampling results, elevated maternal serum AFP, pregnancy complications, and pregnancy outcome. To control for the potentially confounding effect of abnormal karyotype, the analysis also was conducted after all women with abnormal chorionic villus sampling results were excluded. To assess possible clustering of oligohydramnios cases, the study period was divided into quartiles, and the χ^2 test for trend was performed.

Results

A total of 872 women received first-trimester counseling and selected a prenatal diagnosis procedure during the study period. Of these, 477 women chose chorionic villus sampling and 395 chose amniocentesis (amniocentesis group A) (Table I). Women who chose chorionic villus sampling were slightly older than women who selected amniocentesis but did not differ on indications for procedures. Women who underwent chorionic villus sampling were more likely to have bleeding after the procedure and were more likely to have abnormal results than were women who underwent amniocentesis. The number of women who selected neither procedure or who spontaneously aborted after counseling but before either chorionic villus sampling or midtrimester amniocentesis cannot be ascertained through the data set.

There were an additional 2664 women who were seen for amniocentesis in the midtrimester (amniocentesis group B). Their profiles were similar to those of the amniocentesis group A.

Biochemical and chromosomal abnormalities and pregnancy losses to 28 weeks are presented by procedure in Table II. The chorionic villus sampling group had higher rates of spontaneous abortion, midtrimester oligohydramnios, chromosomal anomalies, and metabolic disorders than did either amniocentesis group.

There were three cases of midtrimester oligohydramnios noted among the 2664 women in the amniocentesis group B (0.1%). Two of these cases were associated with chromosomal anomalies (triploidy). Thus, when corrected for chromosomal abnormalities, there was one case of oligohydramnios among 2615 women (0.04%).

In 12 of 442 women (2.7%) with a normal fetal karyotype who underwent chorionic villus sampling, oligohydramnios developed at 16 to 23 weeks compared with none of 391 women with a normal fetal karyotype who were counseled at the same time in pregnancy but elected midtrimester amniocentesis (p = 0.01) (Table III). The differences also were statistically significant when analyzed at 16 to 20 weeks and 20.1 to 23 weeks.

Characteristics of the women undergoing chorionic villus sampling by oligohydramnios status are presented in Tables IV and V. There were no significant differences between these two groups in maternal age, gravidity, parity, number of attempts at chorionic villus sampling, mean gestational age at the time of procedure, or mean amount of villi sampled. Additionally, there was no difference in the prevalence of oligohydramnios between the operators, and the oligohydramnios cases occurred evenly over the study period and were not clustered in any quartile.

Forty-two percent of the women whose pregnancies were complicated by oligohydramnios after chorionic villus sampling experienced bleeding immediately after the procedure, as compared with 8.8% of those with normal amniotic fluid (p < 0.01). Women with oligohydramnios also were more likely to have a maternal serum AFP ≥2.5 multiples of the normal median when compared with those with normal amniotic fluid volume, 16.5% and 1.5%, respectively (p < 0.01). When the analysis was restricted to women at 16 to 20 weeks with normal chorionic villus sampling results, these differences remained significant. Sixty percent of the women with oligohydramnios (3 of 5) had bleeding immediately after chorionic villus sampling, as compared with 9.5% (41 of 435) of those with normal fluid (p < 0.01). Similarly, 40% of the women (2 of 5) with oligohydramnios had elevated maternal serum AFP (>2.5) as compared with 1.6% (7 of 435) of controls with normal fluid volume (p < 0.01).

There were no perinatal survivors among the 12 pregnancies with midtrimester oligohydramnios. The case summaries and pregnancy outcomes are shown in Table VI.

Table I. Pregnancy characteristics of women who had first-trimester counseling: Comparison of women who chose chorionic villus sampling and amniocentesis

	Chorionic villus sampling (n = 477)		gro	ocentesis up A 395)
	No.	%	No.	%
Maternal age (yr)	2			
<20	2	0.6	0	0
20-30	27	5.7	8	2.0
31-40	387	81.2	361	91.4
41-50	60	12.6	24	6.1
Unknown	0	0	2	0.5
Gravidity				
1	51	10.7	81	20.5
2	114	23.9	111	28.1
3	118	24.7	101	25.6
4	97	20.3	55	13.9
≥5	97	20.3	47	11.9
Parity				
0	131	27.5	153	38.7
1	174	36.5	148	37.5
2	126	26.4	77	19.5
3	33	6.9	15	3.8
≥4	13	2.7	2	0.5
Reasons for counseling	400	05.5	0.40	00.4
Advanced maternal age	408	85.5	349	88.4
Previous abnormality	25	5.2	33	8.3
Other	44	9.2	13	3.3
Race	440	00.7	9.00	00.0
White Black	442 1	$92.7 \\ 0.2$	368	$93.2 \\ 0.5$
Other	34	7.1	2 25	6.3
	34	1.1	40	0.3
Uterine position Anteverted	418	87.6	382	96.3
Retroverted	59	12.4	13	3.3
NCH OVELLEG	<u>.</u>	14.4	13	5.5

Comment

Although midtrimester oligohydramnios has been suggested as a complication of chorionic villus sampling,^{8, 9} this has not been systematically evaluated or adequately addressed in the chorionic villus sampling studies to date. We observed midtrimester oligohydramnios in 12 of 442 (2.7%) chromosomally normal pregnancies that experienced chorionic villus sampling compared with 0 of 391 controls who elected amniocentesis-a marked difference. Women who chose chorionic villus sampling were slightly older than controls, but this has been noted in other studies.1,2 Women who chose chorionic villus sampling were more likely to have abnormal results, mostly related to a higher percentage of biochemical indications and to the earlier gestational age at the procedure. They were also more likely to experience after the procedure vaginal bleeding. We observed a higher rate of spontaneous abortion among women who chose chorionic villus sampling than among those who chose amniocentesis, but these rates are not comparable. In our data set we can-

	Chorionic villus sampling $(n = 477)$		grot	Amniocentesis group A $(n = 395)$		Amniocentesis group B (n = 2664)	
•	No.	%	No.	%	No.	%	
Spontaneous abortion Midtrimester oligohydramnios	15 12	3.0 2.7	2 0	0.5 0	9 1	0.3	
TOTAL	27	6.0	2	0.5	10	0.4	
Chromosomal anomalies Metabolic disorders	13 7		4 0		47 0		
TOTAL	20 .	4.2	4	1.0	47	1.8	

Table II. Abnormal results and pregnancy complications by procedure

Table III. Midtrimester oligohydramnios: Comparison of women undergoing chorionic villus sampling and amniocentesis after first-trimester counseling, with normal fetal karyotype

	No. with oligohydramnios					
Length of gestation	Chorionic villus sampling (n = 442)	Amniocentesis group A (n = 391)	- 1			
16-20 wk 20.1-23 wk 16-23 wk	6 6 12	0 0 0	0.03 0.03 0.01			

not identify the number of patients aborting or lost to follow-up after first-trimester counseling but before second-trimester amniocentesis.

Midtrimester oligohydramnios most often occurs with fetal anomalies, either renal or chromosomal, and is extremely rare in otherwise normal gestations. ¹⁰⁻¹² Only one case was observed among 2615 chromosomally normal gestations (0.04%) in amniocentesis group B. The true prevalence could be significantly less than that.

There are at least three potential explanations for the observed differences in oligohydramnios between the two groups. First is the so-called learning curve, which states that complications are highest as new procedures are learned and then decrease as experience is gained. This has been suggested as a reason for increased spontaneous abortion rates among new chorionic villus sampling centers.^{3, 9}

The learning curve effect is supposedly dampened as procedures become more widespread and one learns from the mistakes of others. If the learning curve were a factor, an increased number of catheter insertions, decreased tissue volume, or clustering, either by physician or in any quartile of the study period, might be expected. However, as seen in Tables IV and V, there were no differences in these variables between groups.

Table IV. Pregnancy characteristics of women undergoing chorionic villus sampling: Comparison of women with midtrimester oligohydramnios and normal amniotic fluid volume

		dramnios = 12)	amnio	rmal tic fluid 465)
	No.	%	No.	%
Maternal age (yr)				
≤20	0		3	0.6
>20-≤25	0		7	1.5
>25-≤30	1	8.3	19	4.1
>30-≤35	2	16.7	98	21.1
>35-≤40	5	41.7	282	60.6
≥40	4	33.3	56	12.0
Gravidity				
1	1	8.3	50	10.8
2	5	41.7	109	23.4
2 3	1	8.3	117	25.2
4	4	33.3	93	20.0
≥5 . ,	1	8.3	96	20.6
Parity		•		
0 ′	6	50.0	125	26.9
1	3	25.0	171	36.8
2 3		16.7	124	26.7
3	2 1	8.3	32	6.7
≥4	0		13	2.8
Indication				
Advanced maternal age	10	83.3	398	85.6
Previous abnormality	1	8.3	24	5.2
Other	1	8.3	43	9.2

It also has been further suggested that midtrimester oligohydramnios could result from the chorionic villus sampling catheter indenting the gestational sac, thus causing a disruption. Gestational sac indentation at the time of the procedure was not observed in any of the cases of oligohydramnios in the present series.

A second explanation might be chorionic villus sampling catheter size. The Cook catheter, which measures 5.7 Fr or 20 mm external diameter, was used in the present study. The Portex catheter, the other major chorionic villus sampling catheter, measures 18 mm

Table V. Procedural characteristics of women undergoing chorionic villus sampling: Comparison of women with midtrimester oligohydramnios and normal amniotic fluid volume

	Oligohydran ($n = 12$		Normal amnion $(n = 46)$		
	No.	%	No.	%	p Value
Insertions					
1	11	91.7	383	82.4	NS
2	0	0 .	70	15.0	NS
≥3	1	8.3	12	2.6	- NS
Uterine position	· ·				•
Anteverted	11	91.7	407	87.5	NS
Retroverted	1	8.3	. 58	12.5	NS
Postprocedure bleeding	. 5	41.7	. 41	8.8	< 0.01
Maternal serum AFP ≥2.5 multiples of median	2	16.7	7	1.5	< 0.01
Villi sampled (mean ± SD)	37.6 ± 21.2	• *	29.9 ± 23.1		, NS
Gestational age—procedure (mean ± SD)	10.8 ± 0.8		10.7 ± 0.9	4 F	NS ·
Gestational age—counseling (mean ± SD)	9.8 ± 1.5	•	9.6 ± 1.4		· NS

Table VI. Oligohydramnios and chorionic villus sampling: Case analysis

Case no.	Gestational age (wk)	Tissue volume (mg)	Bleeding	Insertions	AFP (multiples of median)	Gestational age at oligohydramnios (wk)	Outcome
· '1	11.0	50	Yes	1		23	Delivery at 26 wk, neonatal death
2	11.6	28	No	. 1	3.4	18	Delivery at 26 wk, neonatal death
3	11.3	55	No	1		21	Intrauterine fetal death
4	11.5	. 60	Yes	1		16.5	Intrauterine fetal death
5	10.5	. 2	Yes .	3	1.4	22	Intrauterine fetal death
.6	11.7	27	Yes	1	\$ ·	21	Intrauterine fetal death
7	11.0	35	No	- 1,	1.5	22	Delivery at 28 wk, neonatal death
8	11.0	40	No ·	1	**********	15	Voluntary termination of pregnancy
9	10.2	21	No	1	1.5	17	Premature rupture of mem- branes, spontaneous abortion
10	9.4	, 78	No	1	7.0	17	Premature rupture of mem- branes, spontaneous abortion
11	9.6	25	No	1	*	- 19	Intrauterine fetal death
12	10.3	40	No .	. 1	2.0	22	Intrauterine fetal death

external diameter. Most series of chorionic villus sampling data presented to date have used the Portex catheter. The larger catheter size could potentially be related to oligohydramnios, but tissue volumes obtained in this study were similar to those obtained in other studies^{1,5} using other catheters.

A third explanation for the observed differences would be underreporting of oligohydramnios. Without a very careful follow-up protocol that called specifically for midtrimester ultrasonography, several cases of oligohydramnios might have been overlooked. Six of the 12 cases presented as intrauterine fetal deaths and two additional cases had premature rupture of the membranes and spontaneous abortion. Thus two thirds of the cases of midtrimester oligohydramnios could have been missed without careful surveillance. Other studies also have noted late pregnancy losses after chorionic villus sampling that may be associated with oligohydramnios.^{2, 3}

We believe the most likely explanation is that cho-

rionic villus sampling may cause a placental or endometrial injury that may result in perigestational hemorrhage. These changes may result in severely altered fetoplacental perfusion and, over time, midtrimester oligohydramnios. Oligohydramnios was associated with postprocedural bleeding and elevated maternal serum AFP in the midtrimester, both consistent with placental injury. The association with elevated maternal serum AFP may be even stronger than we observed. One half of the cases of oligohydramnios did not have maternal serum AFP determined, presumably because of midtrimester intrauterine fetal death recognized before scheduled blood drawing.

The prognosis for midtrimester oligohydramnios is grim. There were no survivors among the 12 cases. Only three of 12 (25%) achieved 26 weeks' gestation. These three infants all died, two shortly after delivery and the third at 9 months of age, having never left the neonatal intensive care unit.

Our study suggests that first-trimester transcervical

chorionic villus sampling may be associated with an increased risk for midtrimester oligohydramnios and its grim prognosis. Future studies of chorionic villus sampling should be designed to address this issue, and midtrimester ultrasonography should be considered in women who undergo chorionic villus sampling.

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Intrapartum course of fetuses with isolated hypoplastic left heart syndrome

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Once considered universally fatal, the hypoplastic left heart syndrome is now being surgically treated in the newborn period. To help formulate an appropriate management plan for the labor and delivery of these patients, we reviewed the intrapartum course and immediate neonatal outcome of 13 fetuses with known hypoplastic left heart syndrome. Eleven of 13 patients underwent labor, and only one had an abnormal fetal heart rate pattern. There were no cases with meconium staining of the amniotic fluid. All patients with spontaneous or induced labor were delivered vaginally. There were no Apgar scores <8 at 5 minutes, and all umbilical cord blood pH values were ≥7.20. All infants survived to undergo initial reconstructive surgery. We conclude that labor does not appear to be a high-risk situation for the fetus with this disorder. Routine intrapartum fetal heart rate monitoring can be used, oxytocin can be used as indicated, and cesarean section should be reserved for traditional obstetric indications. (AM J OBSTET GYNECOL 1991; 165:1068-72.)

Key words: Hypoplastic left heart, congenital malformations, labor, intrapartum monitoring

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With improved imaging technology and increased use of obstetric ultrasonography, prenatal identification of congenital heart disease is increasing in frequency. At the same time advancements in pediatric cardiac surgery have improved the long-term outlook for many newborns with structural heart defects. Perhaps most dramatically, neonates with left ventricular outflow tract obstruction caused by the hypoplastic left heart syndrome, once considered a universally fatal condition, are now being successfully treated with both reconstructive surgery1 and transplantation.2 In general, newborns with this syndrome are considered to be candidates for surgical treatment if no other significant structural abnormalities are present, the infant has a normal karyotype, and there is no evidence of cardiac failure. The reconstructive surgical technique is a multistage procedure, currently with a 61% 1-year survival after the first palliative procedure and a 40% overall long-term survival after the second reconstructive surgery.3 The present actuarial 3-year survival after cardiac transplantation therapy for hypoplastic left heart syndrome is reported to be 80%.4

Knowledge of an abnormal fetal condition can significantly alter the management scheme for pregnancy and delivery. While some data exist regarding the natural history of hypoplastic left heart syndrome in the fetal and newborn periods, little information is available on the behavior of these fetuses during labor and delivery.5, 6 To help formulate an appropriate intrapartum management plan for the patient with a fetus that is a potential candidate for repair of critical left ventricular outflow tract obstruction, we reviewed the course of labor and immediate neonatal outcome of 13 such patients referred to our center for obstetric and neonatal care.

Material and methods

The prenatal office and hospital charts, including the complete intrapartum fetal heart rate (FHR) monitor tracings, were available for review for 13 fetuses with the antenatal diagnosis of isolated hypoplastic left heart syndrome who were transferred to our institution for obstetric and neonatal management between May 1986 and August 1990. All fetuses were considered to be candidates for neonatal surgical repair; all were free of other structural malformations, had a normal karyotype, and had no ultrasonographic evidence of heart failure. Fetuses who failed to meet these criteria were not considered appropriate surgical candidates and were not accepted in transfer for antenatal management. In each of the 13 cases the identification of structural heart disease was made by the referring physician; the diagnosis of hypoplastic left heart syndrome was verified by a pediatric cardiologist (J.C.H.) after maternal transport with two-dimensional Doppler echocardiography. Antepartum and intrapartum care was provided by a team of perinatologists and obstetric residents. Patients in labor had continuous electronic fetal monitoring, and those who underwent elective cesarean delivery were monitored for at least 30 minutes before establishment of anesthesia. Apgar scores were assigned by pediatricians in the delivery room. Umbilical cord blood pH was measured in 10 of the 13 cases. Two-dimensional Doppler echocardiography was repeated in the immediate neonatal period for diagnostic confirmation. All FHR tracings were reviewed by three of the authors (G.M.J., J.L., A.J.C.) without knowledge of fetal intrapartum course or outcome, and standard criteria were used to interpret the FHR patterns.7

Results

The antenatal identification of hypoplastic left heart syndrome was made at least 2 weeks before delivery in each case, at a mean gestational age of 25 weeks. All 13 of the fetuses were diagnosed as having the hypoplastic left heart syndrome, each had a normal karyotype demonstrated by analysis of either cultured amniocytes or fetal lymphocytes, and none had any ultrasonographic evidence of other major structural malformations or hydrops fetalis.

The average gestational age at delivery was 38.8 weeks (range, 34.5 to 41.5 weeks). Eleven infants (85%) were delivered after 37 completed weeks, one child was delivered at 36 weeks after a pregnancy complicated by premature labor, and one child was delivered at 34.5 weeks because of active labor with a previous classic cesarean section.

All fetuses were in cephalic presentation at the time of onset of labor or planned delivery. Labor was spontaneous in five patients (38%) and electively induced in six (46%). A scheduled repeat cesarean section was performed in one patient (8%), and another cesarean section was performed in a patient in active labor who was not considered a candidate for labor because of a previous vertical uterine incision. Of the five patients with spontaneous labor, one (20%) required oxytocin augmentation. All labor inductions were elective and initiated with oxytocin infusion; because of cervical status, preinduction cervical ripening with prostaglandin gel was not necessary in any instance. All patients with spontaneous or induced labor were delivered vaginally. There was only one operative vaginal delivery, a low vacuum extraction after maternal exhaustion in the second stage of labor.

Of the 11 laboring patients, three had external electronic fetal monitoring only and eight had fetal scalp electrodes applied. Each of the 11 fetuses had a normal baseline FHR, and 10 (91%) had FHR reactivity. No fetal dysrhythmias were noted. Mild or moderate variable decelerations were noted at some point during labor in six cases (55%), and recurrent late decelerations were seen in only one (9%) who had a normal scalp pH (7.31). No fetus manifested severe variable FHR decelerations, and there was no meconium staining of amniotic fluid in any case.

Seven laboring patients underwent epidural anesthesia, and four tolerated labor without analgesia. Two of the patients without epidural anesthesia had local perineal anesthesia for delivery. Both cesarean sections were performed with the patients under regional anesthesia, one spinal and one epidural. There were no anesthesia-related complications.

The range of birth weights was 2180 to 4110 gm (mean, 3042 ± 652). Twelve of the 13 infants had 1-minute Apgar scores ≥ 7 , and all 13 had 5-minute Apgar score of 4, with a 5-minute score of 8 and a venous cord blood pH of 7.36 after an uneventful vaginal delivery. All umbilical cord arterial blood pH values were ≥ 7.20 , and all venous samples had a pH ≥ 7.26 .

All newborns were admitted directly to the neonatal intensive care nursery, and an intravenous infusion of prostaglandin E₁ was started, with elective ventilatory control of acid-base balance used as indicated. All 13 neonates had the diagnosis of hypoplastic left heart syndrome confirmed on the first day of life. Surgical repair was planned for all of the infants in this series, as cardiac transplantation was not available in our center at that time. All of the infants survived to stage I surgical reconstruction, performed between the first and nineteenth day of life (mean, fifth day of life).

Comment

The hypoplastic left heart syndrome is a group of structural cardiac malformations with a hypoplastic or absent left ventricle, a stenotic, hypoplastic, or atretic aortic valve, and a hypoplastic ascending aorta. Approximately 85% of cases have an associated mitral underdevelopment, while the remaining 15% have a common atrioventricular canal with a misalignment of the ventricular septum giving predominance to the right ventricle. Estimated to occur once in every 3000 to 6000 live births, hypoplastic left heart syndrome accounts for approximately 5% to 10% of congenital heart disease and has been cited as the most frequent cause of death from congenital cardiac disease in the first week of life.

The underlying etiology of hypoplastic left heart syndrome is poorly understood, but the fundamental defect is thought to involve interruption of blood flow through the left ventricle, with restriction of either inflow or outflow.8 When not associated with a specific syndrome or chromosomal abnormality, hypoplastic left heart syndrome has traditionally been believed to be multifactorially inherited,8 with an empiric recurrence risk of 0.5% to 2%.11 More recent data challenge the idea of multifactorial inheritance and suggest a much higher incidence of cardiac malformations in first-degree relatives of children affected with hypoplastic left heart syndrome. An evaluation of the siblings of 50 infants with isolated hypoplastic left heart syndrome revealed congenital structural heart disease in 13.5%.12 All of the identified defects were etiologically related to hypoplastic left heart syndrome, being associated with abnormal embryonic blood flow (hypoplastic left heart syndrome, coarctation of the aorta, perimembranous ventricular septal defect). As the multifactorial model does not seem to fully explain the inheritance of isolated hypoplastic left heart syndrome and related malformations, it has been proposed that hypoplastic left heart syndrome is inherited in an autosomal recessive fashion. While simple mendelian inheritance seems improbable, it cannot be statistically rejected on the basis of the available data. 8, 12

The unique physiologic characteristics of hypoplastic left heart syndrome are a consequence of the abnormal anatomy. In utero, blood entering the left atrium is shunted to the right atrium through the foramen ovale. The right ventricle, then, supports the systemic circulation through the ductus arteriosus communicating with the descending aorta. In most cases, right ventricular output is adequate for normal fetal growth. After birth, however, hypoplastic left heart syndrome is said to be a ductal-dependent malformation; that is, systemic perfusion in the newborn period requires atrial left-to-right shunting of oxygenated blood with a patent ductus arteriosus providing a conduit for right ventricular output to reach the aorta. Nearly all infants appear normal until the time of physiologic closure of the ductus, at which time systemic blood flow is interrupted. The timing of the subsequent decompensation with shock and metabolic acidosis is variable but the decompensation usually takes place by the third day of postnatal life. Continued hypoperfusion and worsening acidosis ultimately result in death, which occurs in the first month of life in more than 60% of untreated neonates, with fewer than 5% surviving 1 year. 10, 14

For newborns with hypoplastic left heart syndrome who are candidates for surgical treatment, clinical stabilization requires maintenance of a patent ductus arteriosus and a careful balance between systemic and pulmonary resistance. Ductal patency is most often preserved with a continuous intravenous infusion of prostaglandin E₁. Because ductal closure is a gradual process and does not occur immediately at the time of birth, the prostaglandin infusion need not be initiated in the delivery room but should be started within the first hour or so of life. Elective intubation and paralysis can be used to mechanically hypoventilate these newborns, as a Pco2 of 45 to 50 mm Hg results in an elevated pulmonary resistance, lowered systemic resistance, and improved peripheral perfusion. Mild hypoxia is well tolerated, whereas hyperoxia is to be avoided because of untoward effects on peripheral resistance.3,4

In the fetus with hypoplastic left heart syndrome, real-time ultrasonographic imaging of the heart in the plane of the four-chambered view fails to show the apex of both ventricles at the same level.¹⁵ Confirmation of

the diagnosis in utero requires two-dimensional Doppler echocardiography to completely define the constellation of abnormal findings. In the newborn, qualitative assessment of chamber and vessel location and size are important in affirming the presence of hypoplastic left heart syndrome, and quantitative measurements of left ventricle chamber size have been shown to be predictive of outcome with conditions of severe left ventricular outflow tract obstruction.16 Not only can two-dimensional echocardiography confirm the diagnosis of hypoplastic left heart syndrome, but a complete delineation of cardiac and vascular anatomy is possible. Diagnostic cardiac catheterization was not necessary before surgery in any of our cases, consistent with the experience in other centers.17

Approximately one third of newborns with hypoplastic left heart syndrome have an associated chromosomal abnormality, genetic defect, or major extracardiac structural malformation.9, 14 Chromosomal aneuploidies, most commonly trisomy 18, trisomy 13, trisomy 21, and monosomy X, have been reported in 4% to 11% of cases of hypoplastic left heart syndrome. Additionally, it is seen with a number of other genetic disorders (both autosomal recessive and dominant) and malformation syndromes such as the DiGeorge sequence and the VATER (vertebral defects, imperforate anus, tracheoesophagal fistula, and radial and renal dysplasia) association. Even in the absence of a chromosomal abnormality or malformation complex, the incidence of noncardiac structural malformations concurrent with hypoplastic left heart syndrome is approximately 10%.18-20 Although rare, nonimmune hydrops fetalis has been reported with hypoplastic left heart syndrome²¹⁻²³; structural heart disease with fetal hydrops is a very poor prognostic sign, with nearly universal mortality in some series.23

It should be stressed that our patients represent a highly selected group. All had the diagnosis of a specific congenital heart malformation made in utero and had follow-up in a high-risk setting with an assiduous search made for associated structural and chromosomal abnormalities. Thus differences in our results from those of earlier reports are not unexpected. In a retrospective review of the intrapartum FHR tracings of 16 fetuses with major cardiovascular anomalies, Garite et al.5 found a 12.5% incidence of abnormal FHR baseline, a 31% incidence of absent FHR reactivity, and a 37.5% incidence of either persistent late decelerations or severe variable decelerations. This group of patients had a heterogenous mix of cardiac defects, many with abnormal karyotypes and other associated abnormalities.

Moodie et al.6 briefly outlined the pregnancy outcome of 11 newborns with hypoplastic left heart syndrome diagnosed after decompensation in the neonatal period. Eight of the 11 had vaginal deliveries, and three

underwent elective cesarean section. Meconium staining of the amniotic fluid was present in 2 (18%). Apgar scores at 1 and 5 minutes were >7 in nine of the 11 infants; the scores of the other two newborns were not reported. No mention is made in their report of anesthesia for labor and delivery, oxytocin use, intrapartum monitoring, FHR patterns, or umbilical cord blood pH measurements. This series is relatively homogenous in that all of the neonates had hypoplastic left heart syndrome, but all cases were diagnosed in the newborn period, and the focus of the report is from a viewpoint of pediatric cardiology with a paucity of information about the antepartum and intrapartum courses.

The antepartum management of a patient with a fetus with hypoplastic left heart syndrome should include a consideration of abortion if the diagnosis is made before the legal gestational limit for pregnancy termination. If abortion is chosen, the delivered fetus should have a full autopsy and chromosomal analysis, as any association of hypoplastic left heart syndrome with a karyotypic abnormality or syndrome will significantly alter the risk and counseling for subsequent pregnancies. For those with a later diagnosis or a strong desire to continue the pregnancy, obstetric managment should be guided by the results of fetal karyotype analysis and a targeted ultrasonographic survey of fetal anatomy; a policy of nonintervention on fetal behalf seems justified in cases with a chromosomal abnormality or other major structural malformations. Additionally, serial ultrasonographic evaluations in the third trimester seeking evidence of heart failure are warranted, because fetal hydrops may not manifest early in pregnancy and can significantly alter the outlook for survival.

Antenatal transport to a center providing cardiac surgical services has been reported to result in an improvement in the preoperative condition of newborns with hypoplastic left heart syndrome, with decreased incidences of metabolic acidosis and preoperative cardiac arrest.24 If surgical correction of the cardiac defect is anticipated, delivery in a tertiary care center providing pediatric cardiac surgical services should be arranged. If this is not possible and delivery is to occur in a location remote from the site of the planned repair, consultation with both a neonatologist and a pediatric cardiologist should be obtained well before the time of delivery to ensure the availability of appropriate care immediately after birth and to arrange newborn transport.

The results of our series suggest that the intrapartum period is not a high-risk situation for the fetus with hypoplastic left heart syndrome who is a surgical candidate, free of genetic abnormalities, coexisting major structural malformations, and cardiac failure. Labor, both spontaneous and induced, was well tolerated in our patients with no increased occurrence of FHR abnormalities, fetal distress, meconium staining, acidosis, or neonatal depression, compared with established rates for fetuses without cardiac abnormalities.7 Thus it seems that no special intrapartum precautions or monitoring are necessary. FHR assessment with either external cardiotocography or a scalp electrode is appropriate, with further evaluation of fetal status (scalp stimulation or blood sampling) being performed for standard indications.25 Elective abdominal delivery is not indicated for this group, and cesarean section should be reserved for customary obstetric conditions. Perinatal specialists should be involved in the antepartum management of these patients, and immediate neonatal care is crucial. Although delivery should take place in a tertiary care center, specialized high-risk care does not seem to be necessary for labor and delivery.

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Fetal movement during labor

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The prognostic significance of antepartum fetal movement is well known; therefore it may be a variable in intrapartum fetal well-being. We report the simultaneous observation of fetal movement with fetal heart rate and uterine contractions by processed Doppler actograph signals during spontaneous labor of 22 normal women with normal fetal outcome. The mean percent incidence of fetal movement during labor was 17.3%. The percentage occurring during uterine contractions was 65.9%. Of all uterine contractions, 89.8% were associated with fetal movement. The proportion of time the fetus spent moving during uterine contractions (21.4%) was higher than between uterine contractions (12.9%). Uterine contractions associated with fetal movement were significantly longer than those not associated with fetal movement (p < 0.0001). Mean percent incidence of fetal movement did not differ significantly between latent and active-phase labor. This study demonstrates a clear relationship between fetal movement and uterine contractions in labor. (AM J OBSTET GYNECOL 1991;165:1073-6.)

Key words: Fetal movement in labor, Doppler actograph, fetal well-being

The presence of fetal heart rate (FHR) accelerations associated with fetal movement is the basis for antepartum nonstress testing, and the presence of coexisting fetal movement during the third trimester is indicative of fetal well-being.1,2 Animal studies have demonstrated the cessation of fetal body movements as an initial response to hypoxia.3 Therefore the absence of fetal activity may be a marker for hypoxia during labor in the human fetus. The few studies that have observed fetal movement during labor have done so with ultrasonography for only a small proportion of the entire course of labor. 4-10 In spite of their limitations, these studies suggest both the presence of fetal movement during labor and a significant correlation of fetal movement with uterine contractions. The purpose of this observational study was to quantitate fetal movement during the entire course of the normal labor process with a Doppler transducer capable of simultaneous detection of fetal movement and FHR, coupled with standard measurements of uterine contractions.

Material and methods

Twenty-two low-risk pregnant women with singleton fetuses with vertex presentation who were seen in spontaneous labor at the Johns Hopkins Hospital between

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Reprint requests: Timothy R. B. Johnson, MD, Director, Maternal-Fetal Medicine, The Johns Hopkins University, Houck 228, 600 N. Wolfe St., Baltimore, MD 21205. 6/6/30947 May and July 1990 were eligible for study. Maternal ages ranged from 15 to 31 years, with gestational ages between 36 and 42 weeks. All had essentially benign prenatal courses: One had sickle cell trait, none were illicit drug users (negative toxicology screens), the majority took no medications during pregnancy (four took short courses of antibiotics, two required terbutaline because of preterm contractions), and they were generally nonsmokers (two women smoked five or fewer cigarettes per day). All had adequate weight gain during pregnancy (between 21 and 55 pounds). The study group comprised 12 black and 10 white women.

After these women were determined to be in labor (more than three uterine contractions per 10 minutes or rupture of membranes), informed consent for participation was obtained. All received epidural anesthetic between 2 and 9.5 cm cervical dilation. Eight required oxytocin augmentation. In eight women membranes ruptured spontaneously, whereas the remaining 14 had membranes ruptured artificially.

All 22 fetuses had normal outcomes with 1-minute Apgar scores ranging from 6 to 9, 5-minute Apgar scores ranging from 8 to 10, and an average umbilical artery pH of 7.29 (one pH was <7.2). Twenty were delivered vaginally and two were delivered by cesarean section (one because of prolonged first stage labor, one because of prolonged second-stage labor with failure of the fetus to descend). The average birth weight was 3379 gm, with a range from 2600 to 4060 gm.

A Toitu MT-322 fetal monitor was used to obtain information on FHR, fetal movement, and uterine contractions during labor. FHR signals were processed in the usual manner with autocorrelation techniques. Fetal movement was measured by a Doppler signal of ≥8 Hz

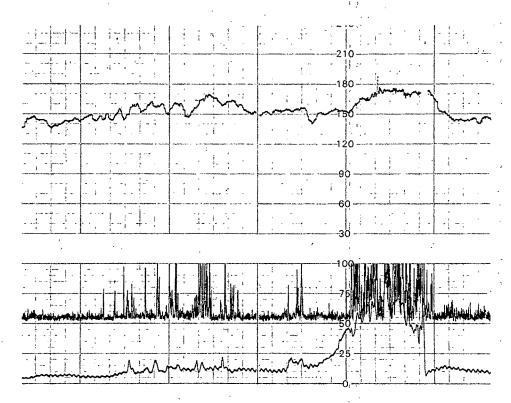


Fig. 1. Fetal monitor tracing showing three channels: top, FHR pattern; middle, Doppler actograph; bottom, uterine contractions. Note simultaneous occurrence of activity with uterine contraction and heart rate acceleration. Paper speed 3 cm/min.

introduced into a 20 to 80 Hz band-pass filter.¹¹ Previous work has related amplitude of recording to type of movement, with peaks >30 arbitrary units corresponding to fetal movement.^{11, 12} Uterine contractions were simultaneously recorded by the same monitor by external tocography or an intrauterine pressure catheter.

Once fetal surveillance with the Doppler actograph was started, the principal investigator (U.R.) supervised the fetal monitor tracing. Continuous tracings were obtained throughout labor, excluding time periods of interference because of maternal movement, vaginal examinations, epidural catheter insertion, and misplaced Doppler or tocography devices.

For study purposes fetal movement recorded at >30 units was used for evaluation (Fig. 1). Fetal movement was then grouped into epochs (period of movement lasting ≥2 seconds) for easier evaluation. The end of an epoch occurred when there was no fetal movement for >4 seconds. The duration of each epoch was considered in 2- to 4-second intervals (e.g., an epoch lasting >6 and <10 seconds would be rounded off to 8 seconds; an epoch >42 and ≤46 seconds would be 44 seconds). A single movement, <2 seconds in duration (likely an isolated limb activity) was counted as 1 second of movement. Therefore the duration of fetal movement was the summation of the duration of the epochs plus the duration of the isolated limb movements.

Fetal movement was further subdivided as not associated with uterine contractions and occurring between 10 seconds before the start of a uterine contraction and 10 seconds after the end of a uterine contraction. Uterine contractions were defined as an increase in uterine tone with a peak of >40 mm Hg and lasting a minimum of 40 seconds. Uterine contractions were divided as not accompanied by fetal movement and accompanied by fetal movement.

Finally, the labor process was divided into a latent phase (0 to 4 cm cervical dilatation) and an active phase (>4 cm dilatation). Individual Friedman curves were plotted to determine the probable time at which 4 cm had been reached.

The SYSTAT statistical package was used to perform paired *t* tests on the sample data. The incidences of the following were compared: fetal movement with and without uterine contractions, uterine contractions with and without fetal movement, and percent fetal movement in the latent phase and the active phase of labor.

Results

A total of 6885 minutes (114 hours) of monitor tracing during labor on 22 healthy fetuses was recorded. After exclusion of poor observation time associated with maternal movement, vaginal examinations, epidural insertion, and misplaced Doppler and tocography devices, 6052 minutes (100 hours) were evaluated. The

average duration of fetal movement constituted 17.8% of the recording period (range, 2.1% to 47.5%; SD, 11.4%).

The total durations of fetal movement without and with uterine contractions were 34.1% and 65.9%, respectively (p < 0.001). The percentage of fetal movement occurring during uterine contractions was significantly higher (Table I). The proportion of time the fetus spent moving during uterine contractions was significantly higher than between uterine contractions, 21.4% and 12.9%, respectively (p < 0.001).

A total of 1689 uterine contractions were recorded, occupying 3140 minutes, or 51.9%, of the recording period. Fetal movement accompanied 89.9% of the contractions, and 10.2% of the contractions were not accompanied by fetal movement (p < 0.001). The mean durations of uterine contractions without and with fetal movement were 65.8 and 117.0 seconds, respectively (p < 0.001). Therefore uterine contractions accompanied by fetal movement were significantly longer than those not accompanied by fetal movement.

In nine women the mean percentage incidence of fetal movement (the percentage of observation time during which the fetus was moving) in latent-phase and active-phase labor was compared. There was no decrease in the mean percent incidence of fetal movement as labor progressed.

Comment

Study findings concur with previous studies demonstrating fetal movement during labor. This study is unique in that fetal movement was conveniently monitored throughout the labor process concurrently with uterine contractions and FHR with the Doppler actograph. Previous studies have established the importance of antepartum fetal movement. Sadovsky and Yaffe¹³ showed that pronounced decreases in movement, culminating in the cessation of fetal movement, occurred in the terminal stages of fetal death in utero. Roberts et al.14 found that the incidence of fetal movement during the third trimester was 18%. Carmichael et al.15 found that before spontaneous labor at term there is a normal decrease in the incidence of fetal breathing movements but no similar change in the incidence of gross fetal body movements. Therefore the presence of gross fetal body movements is a more consistent index of fetal health before spontaneous labor

Boylan and Lewis¹⁶ observed a reduction, although not a statistically significant one, in the fetal movement index (percentage of observed time during fetal trunk movement) from the antepartum period to labor. Richardson et al.6 observed that the intermittent patterns of increased body movement and heart rate variability continued throughout the first stage of labor in spite of the decrease in fetal breathing activity during latent-

Table I. Percent fetal movement time in labor

Labor (mean)	17.3
Between uterine contractions	12.9
During uterine contractions	21.4

and active-phase labor. However, they found a decrease in fetal body movements, possibly related to the progress of labor. Similar findings were reported by Yarkoni and Hobbins,5 who demonstrated that the mean incidence of fetal movement in labor was 19.5% and that there was a linear decrease in percent incidence of fetal movement as cervical dilatation increased. This study found a mean percentage incidence of fetal movement similar to that of Richardson et al.6 and Yarkoni and Hobbins,5 indicating fetal movement to be a stable parameter at the onset of and throughout the labor process. Although observed only in a limited number of women, we noted no decrease in the percentage incidence of fetal movement as cervical dilation progressed. However, further study is required.

Similar to Sadovsky et al.8 and Zimmer et al.,4 we found a close relationship between uterine contractions and fetal movement. We determined an incidence of fetal movement without and with uterine contractions, of 31.8% and 66.2%, respectively, similar to the study of Zimmer et al. However, we found a significantly greater number of uterine contractions associated with fetal movement (89.8% of all uterine contractions). This is probably because of the greater sensitivity of the Doppler actograph apparatus for detecting isolated limb movements and the large number of uterine contractions that were evaluated. However, the effect of epidural anesthesia and oxytocin may have influenced these results, and further study is required on their effects.

Few studies have examined the implications of intrapartum fetal movement monitoring. Wittmann et al.7 concluded that absence of any fetal activity for more than 45 of the 60 minutes of observation during labor is significantly correlated with an abnormal FHR throughout the course of labor. During labor a healthy fetus has short episodes of respiratory movements and reacts to contractions with bursts of movement that are associated with an increase in the FHR. Decreased total activity may indicate a fetus at risk. Chr et al.17 found that fetal reactivity-not only antepartum but in the first stage of labor-is a reliable indicator of fetal wellbeing. They concluded that a nonreactive FHR pattern (lack of accelerations within 20 minutes of admission to the hospital for labor) during first-stage labor should arouse suspicion of intrauterine fetal compromise.

The Doppler actograph in conjunction with traditional FHR and uterine contraction recording is a convenient method for intrapartum fetal surveillance. This study demonstrates the presence of movement of healthy fetuses throughout labor and a clear relationship between fetal movement and uterine contractions. Ultimately, the Doppler actograph will allow us to evaluate fetal behavioral states during labor and determine the clinical implications of fetal movement during labor.

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Editors' note

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case reports, and brief communications articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Oligohydramnios: Antepartum fetal urine production and intrapartum fetal distress

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Animal and human studies suggest that fetal oliguria is a normal physiologic response to hypoxemia. To assess the clinical significance of this observation, we studied (before their admission) 51 fetuses of women whose pregnancies were complicated by oligohydramnios at ≥38 weeks' gestation. We found that the mean hourly fetal urine production decreased significantly in relation to the severity of subsequent intrapartum fetal compromise. The mean antepartum rate was 95 ml/hr in the 21 fetuses with a normal intrapartum heart rate pattern; this fell to 59 ml/hr in the 18 fetuses who had an abnormal intrapartum heart rate pattern but who responded to intrauterine resuscitation. The rate was 33 ml/hr in the 12 fetuses who were delivered by cesarean section as a result of fetal distress. These findings suggest that oligohydramnios associated with fetal oliguria may be used to identify those fetuses who have less intrinsic or uteroplacental reserve than do those of women with oligohydramnios who have a higher rate of fetal urine production. (Am J Obstet Gynecol 1991;165:1077-80.)

Key words: Oligohydramnios, fetal urination, fetal distress

The relationship between decreased amniotic fluid volume and adverse perinatal outcome¹⁻⁵ has led to the assessment of amniotic fluid volume as an integral part of antenatal fetal surveillance programs.⁵⁻⁷ Although a semiquantitative estimate of amniotic fluid volume is simple and rapid, there is currently no universally accepted definition of oligohydramnios. In 1981 Manning et al.¹ diagnosed oligohydramnios when the largest pocket of amniotic fluid was ≤1 cm in its greatest dimension. Chamberlain et al.³ later suggested that this criterion should be changed to ≤2 cm. Subsequently, a number of other semiquantitative methods^{8, 9} were proposed in an effort to improve the predictive value of a reduction in amniotic fluid volume.

Not only is there disagreement about what constitutes a clinically significant reduction in the amount of amniotic fluid, but the multiple pathways by which amniotic fluid is produced and the ways in which amniotic fluid volume is maintained ¹⁰⁻¹² make it difficult to determine the causes of oligohydramnios in every case. If no abnormalities exist in the genitourinary tract, oligohydramnios is often the result of a hypoxemia-induced reduction in renal blood flow that impairs fetal urine production. ¹³ Observations of the newborn infant

support the premise that perinatal asphyxia, in which a decreased rate of urine production may be present, adversely affects renal function. ^{14, 15} Additional support for this concept was recently provided by Nicolaides et al., ¹⁶ who reported a significant correlation between low hourly fetal urine production rates and fetal hypoxemia. In addition, animal studies ^{17, 18} suggest that fetal oliguria may be a normal physiologic response to hypoxemia.

However, because of the many possible pathways for amniotic fluid production and removal, other factors that are unrelated to hypoxemia may be responsible for the observed decrease in amniotic fluid volume. Of the possible fluid exchange routes, only the rate of fetal urine production can be assessed in utero by noninvasive means. ¹⁹ Because a hypoxemia-mediated decrease in fetal urine output may be responsible for a significant number of cases of oligohydramnios, it is reasonable to postulate that the condition is a disorder of varying severity and that those cases that arise as a result of fetal hypoxemia may be identified by a decrease in the rate of fetal urine production.

This study was designed to assess perinatal outcome in terms of fetal urine production in patients with oligohydramnios at ≥38 weeks' gestation. If a decrease in fetal urine production before the onset of labor is a result of unrecognized chronic hypoxemia in patients with oligohydramnios, then the fetuses of these patients may have an increased risk of experiencing fetal distress during labor.

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Material and methods

Approximately 200 obstetric ultrasonographic examinations are performed each week by four experi-

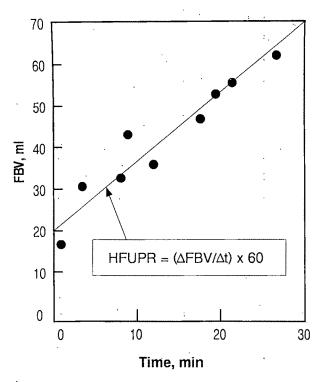


Fig. 1. Fetal bladder volume (FBV) in milliliters as a function of time (in minutes) for single fetus. Circles denote clinical measurements and solid line is least-squares best fit; slope of this line is rate of fetal urine production (HFUPR) (in milliliters per minute).

enced technicians in the Ultrasound Laboratory at the University of Alabama at Birmingham. A GE RT-3000 is used for all obstetric scans. In this study, which was approved by the institutional review board, diagnosis of oligohydramnios was based on the visual qualitative estimate of amniotic fluid volume, which was assessed by an experienced ultrasonography technician. When patients with oligohydramnios were identified, the semi-quantitative method originally described by Phelan et al.9 was performed and the amniotic fluid index measured was recorded in the computerized data base. This information was not made available for decisions regarding management of patients. Patients with qualitative oligohydramnios were recruited for this study only if the amniotic fluid index was also ≤8 cm. Exclusion criteria included estimated gestational age <38 weeks, an underlying maternal and/or obstetric complication, a nonvertex presentation, a multiple gestation, and any evidence of overt fetal distress at the time of evaluation.

Oligohydramnios was confirmed by one of the authors (C.L.N. or L.J.G.) before the rate of fetal urine production was measured. All measurements of the fetal bladder were made by either C.L.N. or L.J.G. The technique used to measure the hourly fetal urine production rates was similar to that described recently by Rabinowitz et al. 19 Longitudinal and transverse scans of

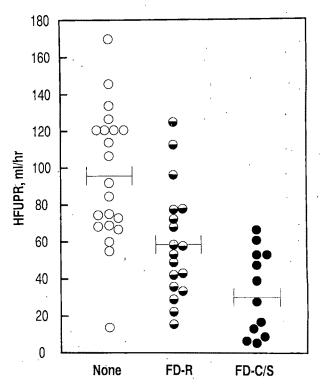


Fig. 2. Hourly fetal urine production rate (HFUPR) for three different outcomes: no fetal distress (open circles), fetal distress responsive to intrauterine resuscitation (half solid circles), and cesarean section because of fetal distress (solid circles).

the fetal bladder were performed at its greatest dimension, and the transverse, longitudinal, and anteroposterior diameters were recorded. The fetal bladder volume was calculated with the equation: Fetal bladder volume = 0.52 × Transverse diameter × Longitudinal diameter × Anteroposterior diameter. Fetal bladder measurements were taken after micturition and were performed at 3- to 5-minute intervals for a period of approximately 30 minutes. The time corresponding to each fetal bladder volume was taken to be the average value of the time recorded at the measurement of the longitudinal diameter and the time recorded at the measurement of the transverse diameter. To estimate the mean hourly fetal urine production rates, a graph of the fetal bladder volume versus time was prepared for each fetus, and the mean hourly fetal urine production rates was then determined from the slope of the line that best fit these data (Fig. 1).

At the University of Alabama at Birmingham, any patient with a term gestation is generally admitted for delivery when the diagnosis of oligohydramnios is made. The mean hourly fetal urine production rates for each study patient was computed in the clinic, usually several days after the patient had been delivered and without the clinic staff's knowing the delivery outcome. In no case was the hourly fetal urine production rate made available to the physicians managing these

patients. In addition, the managing physicians were unaware that the patients were part of an ongoing

Labor and delivery care is provided by the resident staff under the direct supervision of in-house faculty. Fetal distress was considered to be an abnormality of the heart rate pattern (usually persistent late decelerations or baseline bradycardia) that was sufficient to require initiation of fetal resuscitation or an immediate cesarean section. Intrauterine resuscitative efforts included the cessation of oxytocin, an intravenous infusion of crystalloid, placing the patient in a lateral recumbent position or changing the patient's position, and the administration of oxygen. All decisions to perform a cesarean section because of a persistent abnormality in the fetal heart rate pattern were made by the attending faculty.

Results

The study population of 51 gravid patients included 27 multiparous women; the group was predominantly nonwhite (69%). The mean age of the patients was 23 ± 5.7 years, and the mean gestational age at delivery was 41 ± 1.1 weeks (range, 38 to 43 weeks). The mean interval from hourly fetal urine production rate determination until delivery was 0.76 ± 0.95 days (range, 0 to 5 days). Only three of the 51 patients had thick meconium at the time of amniotomy. Cord arterial blood gas data were available on 44 patients. Nine fetuses were considered to have evidence of acidemia (pH < 7.2). Of these nine, four were considered to have a primary respiratory acidemia, and five had either a mixed or indeterminate acidemia.20 Two of these nine were considered to have had reassuring fetal heart tracings, two responded to intrauterine resuscitation, and the remaining five underwent a cesarean delivery because of fetal distress. All 51 neonates have a 5-minute Apgar score >7. According to the criteria of Brenner et al.,21 only one patient was delivered of an infant whose birth weight was <10th percentile, whereas seven were considered to be >90th percentile.

The study population was divided into three groups according to the presence and the nature of intrapartum fetal distress. The control group (n = 21) consisted of fetuses whose intrapartum heart rate pattern was normal. Fetuses who had persistent heart rate abnormalities in the first or second stage of labor but who responded to intrauterine resuscitation or were delivered vaginally formed the second group (n = 18). The third group (n = 12) consisted of patients who required cesarean delivery because of a persistent fetal heart rate abnormality. There was no difference in the mean gestational age among the three groups (p =0.39). The 12 infants who were delivered by cesarean section because of fetal distress had a mean hourly fetal urine production rates of 32 ml/hr, compared with 57

ml/hr for fetuses who responded to intrauterine resuscitation and with 95 ml/hr for fetuses who showed no evidence of distress (Fig. 2). Wilcoxon's rank-sum test indicated that that were statistically significant differences in the hourly fetal urine production rates between the group with no sign of fetal distress and the group who responded to intrauterine resuscitation (p = 0.002) and between the control group and those requiring a cesarean section because of fetal distress (p < 0.001).

Comment

The purpose of this prospective study was to determine whether the rate of fetal urine production is related to the intrapartum fetal heart rate pattern in women with oligohydramnios at term. We found that fetuses who were delivered by cesarean section because of fetal distress had significantly lower hourly fetal urine production rates when compared with fetuses who exhibited no sign of fetal distress during labor (32 vs 95 ml/hr, p < 0.001).

Animal studies suggest that fetal oliguria may be an important component of the fetal adaptive response to hypoxemia. A significant decrease in the rate of fetal urine production has been observed in fetal sheep after partial occlusion of the umbilical cord17 and during maternal hypoxia.18 At least two factors may be responsible for a reduction in fetal urine output in this setting. First, there is a redistribution of the combined ventricular output to maintain oxygen delivery to vital organs: Blood flow to the fetal brain, heart, and adrenal glands is either maintained or increased during hypoxemia, whereas blood flow to less vital organs, such as the kidney, is decreased.^{22, 23} This reduction in renal perfusion results in a decrease in the rate of urine production by the fetus.24 A second factor is an increase in the reabsorption of free water in the distal tubules.18 This effect is probably mediated by antidiuretic hormone,18 because fetal hypoxemia is known to be a potent stimulus for antidiuretic hormone release25 and because antidiuretic hormone infusion produces a decrease in fetal urine output.26

Clearly the effect of the diagnostic criteria used to establish the diagnosis of oligohydramnios must be considered. Our study population consisted of patients who were thought to have a decreased amniotic fluid volume as determined by a technician experienced in ultrasonography. Although an amniotic fluid index ≤5 cm has been suggested as being abnormally low in a postterm population,7 we arbitrarily chose an amniotic fluid index of ≤8 cm as a semiquantitative definition of oligohydramnios in this group of mostly term gestations. Trimmer et al.27 recently reported a decrease in the hourly fetal urine production rates in eight pregnancies at ≥42 weeks' gestation that were complicated by oligohydramnios, in comparison with similar measurements in 30 pregnancies at ≥42 weeks' gestation that had normal amniotic fluid volume. In that study oligohydramnios was diagnosed when the maximum vertical dimension of the largest amniotic fluid pocket measured ≤1 cm. In our study population seven pregnancies were ≥42 weeks' gestation at the time of evaluation; in this small group the mean hourly fetal urine production rates was 67 ml/hr, which is almost three times the rate reported by Trimmer. The reason for this discrepancy is not apparent at this time, although the criteria used to define oligohydramnios differed considerably between the two studies and both studies involved a relatively small number of patients with oligohydramnios at ≥42 weeks' gestation.

We conclude that measurements of the hourly fetal urine production rates may help to identify fetuses with clinically significant oligohydramnios. In our study population a useful definition of fetal oliguria appears to be an hourly fetal urine production rate ≤60 ml/hr. If the end point of a cesarean delivery because of fetal distress is considered, this cutoff has a sensitivity of 83% but a specificity of only 64%. Although these preliminary data are encouraging, larger prospective studies are required to confirm our results and to more fully delineate the predictive value and ultimate clinical use of this finding.

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Antepartum fetal surveillance tests during sickle cell crisis

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A study was conducted to evaluate the characteristics of antepartum fetal assessment tests for women with sickle cell disease during crisis. A total of 24 women with 39 episodes of crisis after 34 weeks of pregnancy were studied. All subjects were evaluated with nonstress tests, biophysical profile score, and uterine and umbilical artery systolic/diastolic ratios during and after sickle cell crisis. Results revealed that the incidence of nonreactive nonstress tests was significantly higher during crisis compared with the period after crisis (58.9% vs 10.3%, p < 0.05). The frequency of biophysical profile score <8 was significantly higher during crisis compared with after crisis (33.3% vs 7.7%, p < 0.05). All subjects had an increase in uterine systolic/diastolic ratio during crisis. The average uterine systolic/diastolic ratio was 3.92 (range, 2.16 to 4.24) during crisis and 2.54 (range, 1.98 to 3.23) after crisis (p < 0.05). In contrast, there was no significant change in the mean umbilical systolic/diastolic ratios during and after crisis (2.58 and 2.62, respectively). We conclude that, although sickle cell disease crisis is associated with a higher incidence of abnormal biophysical test results, in most patients these results will revert to normal after crisis. The increase in uterine vascular resistance without a concordant increase in umbilical vascular resistance suggests that the transient effects of sickling during crisis may not compromise umbilical blood flow. (AM J OBSTET GYNECOL 1991;165:1081-3.)

Key words: Sickle cell crisis, Doppler, nonstress test.

Sickle cell disease is characterized by a number of serious medical problems, including sickle cell crisis, acute chest syndrome, and multiple-organ abnormality.1-5 Painful vasoocclusive crisis is the most common cause of morbidity and the most frequent reason for short-term hospitalization.4 Pregnancy has been associated with an increase in the frequency of painful crises, especially in the last trimester and immediately postpartum. Although fetal surveillance tests such as the nonstress test (NST), contraction stress test, and Doppler studies have been used in the evaluation of pregnancies complicated by sickle cell disease, 5.6 the characteristics of these tests during sickle cell crisis have not been well described previously. The purpose of this investigation was to evaluate the characteristics of antepartum fetal assessment tests for a relatively large group of women with sickle cell disease during crisis.

Material and methods

In a prospective study design, 24 women with sickle cell crisis during the third trimester of pregnancy who attended the prenatal clinic at Albert Einstein College of Medicine between December 1988 and January 1991

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were studied. Diagnosis of acute painful crisis was made by sudden or insidious onset of intense pain involving the abdomen, chest, back, or extremities necessitating hospitalization. After careful evaluation patients were generally treated with hydration and analgesia. Subjects were evaluated with NSTs, biophysical profiles, and uterine and umbilical systolic/diastolic ratios on admission with sickle cell crisis and after the crisis.

The NSTs were performed with patients placed in the left lateral position. Either a model III (Corometrics) or a model 9020 (Hewlett-Packard) fetal monitor with an external tocodynamometer was used.

Fetal movements were documented by the mother using an event marker. A reactive NST was defined as two fetal heart rate accelerations associated with fetal movement in a 20-minute observation period.⁷

A tracing test that did not meet the reactive criteria in an 80-minute period was considered nonreactive.⁸ The criteria for the interpretation of the biophysical profile were those described by Manning et al.⁹ All components of the biophysical profile, including NST, fetal movements, fetal breathing movements, fetal tone, and amniotic fluid volume, were assessed, and a normal result received a score of 2. The maximum biophysical profile score is 10. For some analyses scores were classified as ≥8, <8, and <6.

The diagnostic evaluations to measure uterine and umbilical systolic/diastolic ratios for these women were performed with a continuous-wave Doppler instrument with a 4 MHz transducer. The technique for obtaining both the uterine and umbilical systolic/diastolic ratios has been previously described.⁶ The flow-velocity

Table I. Selected characteristics of study population (N = 24)

	Mean ± SL
Maternal age (yr)	27 ± 6
Nulliparity (%)	62.5
Hemoglobin (gm/dl)	7.9 ± 1.1
Gestational age during crisis (wk)	36.2 ± 2
sestational age during crisis (wk)	30.4

Table II. Distribution of biophysical profile scores during and after crisis

Biophysical profile score	During crisis (n = 39) (%)	After crisis (n = 39) (%)	p Value
≥8	66.3	82.3	
<8	33.3	7.7	< 0.05
<6	5.1	. 2.5	NS

Table III. Frequency of abnormalities in individual biophysical profile parameter during and after crisis

	During crisis $(n = 39)$ $(\%)$	After crisis (n = 39) (%)	p Value
Fetal movement	33.3	7.7	<0.05
Fetal breathing	10.3	7.7	NS
Fetal tone NST	7.7 58.9	5.1 10.3	NS <0.05

NOTE: None of the subjects had decreased amniotic fluid volume. NS, Not significant.

waveforms were quantitated by the ratio of peaksystolic/end-diastolic flow velocity.10, 11

Statistical analyses were performed with χ^2 analysis for categoric data and paired t test for continuous data.

Results

There were 39 documented episodes of sickle cell crisis in the 24 subjects. Selected clinical characteristics of this study population, including maternal age, parity, hemoglobin level, and gestational age, are given in Table I.

The proportion of nonreactive NSTs was significantly higher during crisis compared with the period after crisis (58.9% vs 10.3%, p < 0.05).

The frequency of biophysical profile scores <8 was significantly higher during crisis as compared with after crisis (33.3 vs 7.7%, p < 0.05, Table II). The higher frequency of biophysical profile scores <6 during crisis

as compared with after crisis was not statistically significant.

Evaluations of individual parameters of the biophysical profile revealed that NST and fetal movement were the most frequent abnormalities and were significantly associated with crisis. The proportions for NST and fetal movement were 33.3% and 58.9%, respectively, during crisis compared with 7.7% and 10.3% after crisis (Table III).

All subjects had an increase in uterine systolic/diastolic ratio during crisis. The mean uterine systolic/diastolic ratio was 3.92 (range, 2.16 to 4.24) during crisis and 2.54 (range, 1.98 to 3.23) after crisis (p < 0.05). In contrast, there were no significant changes in the umbilical systolic/diastolic ratios during and after 38 of 39 crises in these women. One patient had abnormality of both uterine and umbilical systolic/diastolic ratios during and after crisis. This was associated with recurrent late decelerations and poor (<6) biophysical profile score. She was delivered by cesarean section of a male infant with Apgar scores of 3 and 6 at 1 and 5 minutes, respectively. The birth weight was 1990 gm at 36 weeks of gestation (<10th percentile for 36 weeks).

Comment

The ability to optimally manage the pregnant woman in sickle cell crisis requires appropriate interpretation of maternal and fetal parameters. The high proportion of nonreactive NSTs during crisis that reverted to normal and the significant difference in fetal activity tests during and after crisis suggest the need to assess all other available information. Interpretation of these findings is facilitated by the proposed mechanism of crisis and disparate results of uterine and umbilical systolic/diastolic ratio.

Vasoocclusive crises are probably due to accumulation of rigid, sickled red blood cells in areas of the microcirculation, causing precapillary and capillary obstruction.12 These events may result in formation of microthrombi and a further decrease in oxygen tension and worsening of the sickling process. The abnormal uterine systolic/diastolic ratio could be explained by the above phenomena, resulting in an increase in uterine vascular resistance. The absence of significant change in the umbilical systolic/diastolic ratio suggests that the events that started on the maternal side did not compromise the fetal circulation. In a previous study6 we showed that adverse neonatal outcomes in pregnancies complicated by sickle cell disease require both maternal and fetal-placental circulations to be compromised, which can be a time-dependent phenomenon. In the current series one patient with abnormally high uterine and umbilical systolic/diastolic ratios required intervention after crisis. This abnormality was associated

with recurrent late fetal heart decelerations. Cesarean section was performed, with delivery of a small-forgestational-age infant having low Apgar scores.

Most investigators agree that a reactive NST has a good negative predictive value13-15; however, concordance has not been demonstrated for women in sickle cell crisis. This study suggests that a nonreactive NST obtained during crisis does not necessarily correlate with increased perinatal morbidity and mortality in the absence of other abnormal findings.

As for the NST, the biophysical profile may be less predictive of outcome in the range of 6 to 8 during crisis, and the strength expected for its predictive potential for adverse outcome may be lessened in these high-risk pregnancies.

Further investigations on larger groups of women with sickle cell disease are needed to understand the mechanisms, assessment modalities, and natural history of crisis in these women. Given the rare incidence of pregnancy in this high-risk population, it is imperative to regionalize management centers and clinical data bases so that these unanswered questions may be resolved.

We conclude that, although sickle cell disease crisis is associated with a higher incidence of abnormal biophysical test results, in most patients these results will revert to normal after crisis. The increase in uterine vascular resistance without a concordant increase in umbilical vascular resistance suggests that the transient effects of sickling during crisis may not compromise. umbilical blood flow.

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Maternal perception of decreased fetal movement as an indication for antepartum testing in a low-risk population

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Maternal perception of decreased fetal movement has traditionally been used as an indication for fetal testing in high-risk patients. Two hundred ninety-two low-risk patients who presented with a complaint of decreased fetal movement were studied to determine the incidence of adverse outcome and need for further testing. Initial testing included a nonstress test and ultrasonographic examination. Five (1.7%) of the study group had fetal death on initial examination and 4.4% of patients required immediate delivery because of abnormal maternal or fetal evaluation. An additional 5.8% had abnormal fetal heart rate tracings that necessitated follow-up. Fifty-two percent of patients with normal initial evaluations underwent additional testing. There were no significant differences between patients undergoing additional testing, patients having no additional testing, and a low-risk nontested group with regard to adverse outcome. Fetal surveillance is indicated in low-risk patients with decreased fetal movement. Additional testing of patients with a normal initial evaluation and no further complaint of decreased fetal movement may not be necessary. (AM J OBSTET GYNECOL 1991;165:1084-8.)

Key words: Decreased fetal movement, low-risk patient, antepartum testing, fetal outcome

Antepartum fetal deaths account for more than half of all perinatal mortality, and in one institution 70% of nonanomalous stillborn infants weighing > 1000 gm resulted from seemingly normal pregnancies.1 Any effort to detect fetal compromise and prevent antenatal mortality may be best served by routine antepartum fetal evaluation. Schifrin et al.2 demonstrated that antepartum fetal testing will predict fetal outcome more accurately than will antenatal risk assessment with an established scoring system. Routine antenatal evaluation of all pregnancies with use of ultrasonography and external fetal monitoring would clearly tax limited resources, but maternal assessment of fetal activity is a simple, inexpensive, and probably effective means of monitoring fetal condition. Sadovsky and Polishuk³ described the "movement alarm signal," a pronounced reduction or cessation of fetal movements or of their vigor for at least 12 hours while fetal heartbeats were still audible. Fetal outcome was improved if delivery was accomplished as soon as this movement alarm signal was observed. These findings have been corroborated by several other authors whose observations were made in patients with identifiable high-risk condi-

tions. 4-10 The clear advantage of maternal surveillance of fetal movement in high-risk patients has led many clinicians to have all their patients monitor fetal movement. The purpose of this study was to evaluate a low-risk patient population whose only complaint was decreased fetal movement for these parameters: (1) the incidence of abnormal fetal test results necessitating immediate intervention, (2) the percentage of patients with incidental abnormal ultrasonographic findings, and (3) the need for long-term follow-up of fetuses with a normal initial evaluation.

Material and method

The Maternal-Fetal Testing Center of the Weiler Hospital of the Albert Einstein College of Medicine serves antepartum patients referred by their private obstetricians. These patients are from a racially mixed, middle- to upper-middle-class socioeconomic background. All patients registered for delivery in our institution were instructed in writing to monitor fetal movement daily and to report any period of decreased fetal movement (fewer than four per hour for two consecutive hours) to their obstetrician. The evaluation of patients presenting with a complaint of decreased fetal movement consisted of confirmation of history of perceived decreased fetal movement by an obstetric nurse, a blood pressure measurement, a urine screen for glucose and protein, a nonstress test (NST), and an ultrasonographic evaluation. The ultrasonographic examination included fetal presentation, placental location and grade, evaluation of amniotic fluid volume, estimated gestational age, and fetal weight, as well as a

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screen for fetal anomalies. An abnormal NST result was followed by biophysical profile scoring.11 Patients who had evidence of maternal or fetal compromise such as elevated blood pressure, late fetal heart rate decelerations, fetal heart rate bradycardia, biophysical profile score <6, or oligohydramnios (a pocket of amniotic fluid <2 cm × 2 cm or an amniotic fluid index value¹² <5 cm) were admitted to the labor floor for further evaluation or delivery. Patients with a nonreactive or equivocal NST, biophysical profile score ≥6, and adequate amniotic fluid were advised to have follow-up examinations as were patients with intrauterine growth retardation, macrosomia, and abnormal placentation or presentation. Additional testing of patients with a normal initial evaluation was at the discretion of the referring physician. The study group comprised all low-risk patients presenting with a complaint of decreased fetal movement during the study period. Six hundred twenty-three consecutive, low-risk, nontested patients who were delivered at the Weiler Hospital during the same period served as a control population. Perinatal morbidity was defined as cesarean section because of fetal distress (defined by either an abnormal fetal heart rate tracing in early labor or an abnormal, scalp pH, whenever possible), an Apgar score ≤6 at 5 minutes, thick meconium, birth weight ≤10th percentile for gestational age, neonatal intensive care unit admission for reasons unrelated to prematurity, or perinatal death. Statistical analysis was performed by means of the χ^2 test; a p value of <0.05 was considered significant.

Results

Of the 4727 patients evaluated in the Maternal-Fetal Testing Center between January 1985 and April 1990, 292 (6.2%) low-risk patients presented with a primary complaint of decreased fetal movement. These patients had no history of medical or obstetric problems and were well up to the time of presentation. The mean maternal age was 28 years (range, 14 to 42 years). Fifty percent of patients were nulliparous, and the neonatal birth weight (mean \pm SD) was 3550 \pm 431 gm. The patients ranged in gestational age from 26 to 42 weeks at the time of initial evaluation. Seventy-six patients (26%) were <36 weeks' gestational age and 17 (5.8%) were >41 weeks' gestational age. All patients underwent initial antepartum testing because of their perception of decreased fetal movement. Sixty-two (21%) patients had some abnormality on initial evaluation that required intervention or follow-up (Table I). Fetal death was diagnosed in 5 patients (1.7%) on initial evaluation. The gestational ages of the patients with fetal death ranged from 33 to 37 weeks; 3 patients were <37 weeks. None of these patients had underlying medical or obstetric problems, and none of the stillborn fetuses

Table I. Initial evaluation of low-risk patients with decreased fetal movements

	n	%
Fetal death on initial examination	5	1.7
Incidental ultrasonographic findings	27	9.2
Admission for immediate intervention	13	4.4
Abnormal fetal heart rate necessitating follow-up	17	5.8
Normal initial examination result	230	78.8

had congenital anomalies. None of the patients who had a live fetus on initial evaluation had fetal death during the subsequent period of observation. These incidental ultrasonographic findings were noted: intrauterine growth retardation, 8 (2.7%); macrosomia, 13 (4.5%); abnormal presentation, 5 (1.7%); placenta previa, 1 (0.3%). There were no fetal anomalies or multiple gestations noted in the study group.

A total of 13 (4.4%) patients were admitted for delivery because of abnormal fetal heart rate, biophysical profile score <6, oligohydramnios, or preeclampsia. Four had intrauterine growth retardation and late fetal heart rate decelerations, 6 had oligohydramnios, and three had severe preeclampsia. They ranged in gestational age from 35 to 41 weeks 5 days and were considered low risk by their private physicians. Five (38.5%) of these 13 patients were ≥41 weeks' gestational age, and these patients accounted for 71% of admissions with oligohydramnios. None of them had undergone testing before presentation with complaints of decreased fetal movement. The cesarean section rate of the patients admitted for delivery was 46%, and all cesarean sections were done because of fetal distress. One neonate was admitted to the neonatal intensive care unit after vaginal delivery. This patient was 35 weeks' gestational age with intrauterine growth retardation and a 40% placental infarction. Apgar scores were 8 and 9 at 1 and 5 minutes, respectively, and the birth weight was 1410 gm (<10th percentile for gestational age). This neonate did well and was eventually discharged home. All other patients admitted for delivery had 5-minute Apgar scores ≥8, and there were no other neonatal intensive care unit admissions.

An additional 17 (5.8%) patients who presented with decreased fetal movement had abnormal fetal heart rate tracings that necessitated follow-up examinations (such as nonreactive NSTs or variable decelerations). One patient in this group was admitted for observation. Gestational age in this group ranged from 29 to 41 weeks. The average number of follow-up visits was 4 (range, 1 to 20). Fourteen patients had vaginal deliv-

Table II. Outcome characteristics of patients with decreased fetal movement with a normal initial result of evaluation versus outcome in low-risk, nontested patients

		Maternal perception movements and normal in	Maternal perception of decreased fetal movements and normal initial evaluation result		
,	Low-risk, nontested $(n = 623)$	No subsequent testing $(n = 107)$	Subsequent testing $(n = 115)$	p Value	
Fetal distress in labor (%)	4.5	2.0	2.4	NS	
Thick meconium (%)	4.0	1.0	1.7	NS	
Low apgar score (%)	1.0	0	0.7	NS	
Neonatal intensive care unit admission (%)	1.5	0	0.7	NS	
Intrauterine growth re- tardation (%)	3	0	0	<0.05*	
Cesarean section because of fetal distress (%)	4.5	4.7	5.2	NS	

NS, Not significant.

eries at term with appropriately grown fetuses. Labor was induced in one patient because of an ultrasonographic diagnosis of intrauterine growth retardation at 37 weeks' gestational age, which was subsequently confirmed by birth weight. Another patient in this group had a nonreactive NST at 31 weeks of gestation. She underwent a total of 10 follow-up examinations. Intrauterine growth retardation was diagnosed by ultrasound examination at 34 weeks of gestation, and at 40 weeks of gestation the fetus had a biophysical profile score of 4 and in addition preeclampsia was diagnosed. Labor was induced and the patient was delivered of a 2690 gm fetus (i.e., intrauterine growth retardation). One cesarean section was performed in this group because of preeclampsia in a term patient; this fetus was appropriately grown. All of the fetuses in this group had 5-minute Apgar scores ≥8 and there were no neonatal intensive care unit admissions.

All but two of the 66 patients who were <37 weeks' gestational age on initial evaluation were delivered at term (one was delivered at 33 weeks and the other, at 36 weeks). One patient had a cesarean section because of preeclampsia at 41 weeks; all 5-minute Apgar scores were ≥8, and there were no neonatal intensive care unit admissions.

Six hundred twenty-three consecutive, low-risk, nontested patients who were delivered at the Weiler Hospital during the same period served as a control population. The mean maternal age was 27 years (range, 15 to 43 years). Forty-three percent were nulliparous, the neonatal birth weight (mean \pm SD) was 3410 \pm 570 gm, and the mean gestational age at delivery was 38.6 weeks. Outcome characteristics of these patients are presented in Table II.

Fifty-two percent of patients with decreased fetal movement and a normal result of initial evaluation underwent additional testing. An average of two examinations per patient was performed with a range of I to 20. There were no significant differences in outcome between patients who underwent additional testing, patients having no additional testing, and the low-risk, nontested control group with regard to fetal distress in labor, thick meconium, low Apgar scores, or neonatal intensive care unit admissions (Table II).

Comment

Fetal movement is one of the first signs of fetal life. The mother first becomes aware of fetal movement (quickening) between the sixteenth and twentieth weeks of gestation. This quickening can assist in calculating gestational age, and when it is present for ≥25 weeks, one can be 90% certain that the fetus is beyond the 38th week of gestation.18 Daily fetal movement increases in vigor and frequency with maximum values detected between the 29th and 38th weeks of pregnancy.3 Two weeks before labor there is a slight decrease in the weekly average of fetal movement.3, 5, 8 The reason for this decrease remains unresolved. It has been suggested that the decrease in amniotic fluid volume coupled with the increased fetal size may explain the decreased movement. Normal fetal activity observed subjectively or objectively is an indication of fetal health, and the absence or diminution in fetal movement may indicate fetal death or impending fetal death.14

Fetal movement recording by the mother is a wellestablished method of antepartum fetal assessment. It is easily used by most patients, and maternal assessment of activity correlates well with values obtained with recording devices. 15, 16 It is inexpensive and noninvasive and is not significantly affected by educational level, obesity, gestational age, or smoking status.16 We have examined a group of low-risk patients who were monitoring fetal activity as an ongoing screening tool. Sixty-

^{*}When low-risk nontested patients are compared with patients with decreased fetal movements and normal initial evaluation.

two (21%) of the patients in the study group had abnormalities necessitating intervention or follow-up, demonstrating the usefulness of maternal assessment of fetal movement. Five (1.7%) patients presented with fetal death. This incidence is much lower than that reported by Leader et al.,8 who evaluated 39 high-risk patients with decreased fetal movements and reported a 38.5% incidence of stillbirth.

The patients in our study group who presented with fetal death on initial examination probably represent patients who suffered acute fetal distress as described by Sadovsky and Polishuk.3 One would probably not be able to salvage these fetuses with maternal evaluation of fetal movement. The fetuses that one might hope to rescue are those that are suffering unsuspected uteroplacental insufficiency with subsequent hypoxia, either long-standing or of gradual onset, as in the patient with intrauterine growth retardation and/or hypertensive disorders of pregnancy. The patients in our study group who required admission for delivery all fell into this category, exhibiting fetal heart rate decelerations, a biophysical profile score <6, oligohydramnios, postdate pregnancy, intrauterine growth retardation, and preeclampsia. Most of these patients had intrapartum fetal distress, a further indication of uteroplacental insufficiency. Fetal morbidity was an associated finding in 8 of the 13 patients who required admission immediately after the initial evaluation. Similarly Liston et al.1 reported "fetal compromise" in 63.6% of their high-risk patients presenting with decreased fetal movement. None of our patients who presented with live fetuses had fetal death during the period of observation. Grant et al.,17 in a large multicenter, multinational prospective study, suggest that there is at best minimal benefit from formalized maternal counting of fetal movement. They observed 17 antepartum fetal deaths in patients who presented with live fetuses but complained of decreased fetal movement. None of these patients were delivered emergently, and it is possible that misleading antepartum testing or suboptimal management contributed to the unhappy outcome in many of these cases.

Twenty-seven (9.2%) of the study patients had incidental abnormal ultrasonographic findings; the most common were macrosomia and intrauterine growth retardation. We did not find any fetal anomalies, but this is probably a result of the relatively small sample size and the fact that most of these private patients had an ultrasonographic examination at 18 to 20 weeks of gestation. All of these ultrasonographic findings were of clinical significance and afforded an opportunity for appropriate management.

Ahn et al.18 suggested that patients with decreased fetal movement, normal NST results, and normal amniotic fluid volume, with no other indication for testing, do not require follow-up testing. Our results concur. Two hundred thirty (78.8%) of the study patients had a normal initial result at evaluation. When patients with a normal initial evaluation and no additional testing were compared with those who had additional testing, there was no significant difference in perinatal outcome. In addition, there was no evidence of increased fetal compromise in the patients who presented with a complaint of decreased fetal movement and had a normal initial evaluation as compared with a low-risk nontested population (Table II). These conclusions are limited, however, by the relatively small sample size, which may result in a type II error, and also because there is no practical way of ascertaining how many patients in the low-risk population actually monitor fetal movement as instructed.

Therefore we conclude that it is prudent to evaluate low-risk patients who present with a complaint of decreased fetal movement. In addition, ultrasonographic examination of these patients may provide useful information. Because of the inability to assess the reliability of individual perception of decreased fetal movement, it seems reasonable that patients who continue to complain of decreased fetal movement undergo follow-up examinations. Further testing of low-risk patients with normal initial results of evaluation and no further complaint of decreased fetal movement probably is not indicated.

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Correlation of amniotic fluid index and nonstress test in patients with preterm premature rupture of membranes

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The amniotic fluid index and the nonstress test are commonly used in the expectant management of preterm premature rupture of membranes. This study was designed to investigate the interrelationship of the nonstress test and the amniotic fluid index during the preterm premature rupture of membranes latency period. Fifty patients with preterm premature rupture of membranes for >48 hours were prospectively followed with daily 1-hour nonstress tests and blinded, daily amniotic fluid index examinations (totaling 422 evaluations). The overall average daily amniotic fluid index was statistically lower in the earlier gestations and nulliparous patients but was not influenced by the fetal position or nonlaboring uterine activity. An increased incidence of variable decelerations and nonreactive nonstress tests was associated with a significantly lower overall average daily amniotic fluid index, but these differences were beyond the standard precision of the amniotic fluid index examination. The daily nonstress test appears to identify clinically significant lower fluid volumes during the latency period and should remain the mainstay in the management of preterm premature rupture of membranes. (AM J OBSTET GYNECOL 1991;165:1088-94.)

Key words: Amniotic fluid index, premature rupture of membranes, nonstress test, expectant management of premature rupture of membranes, fetal evaluation

Premature rupture of the membranes occurs in approximately 10% of pregnancies¹ with a high incidence in the preterm (<37 weeks) gestation. Preterm premature rupture of membranes has been associated with a high degree of maternal and perinatal morbidity and mortality.¹⁴ Over the past 10 to 15 years, conservative, expectant management to achieve prolongation of gestation has been one commonly practiced approach

careful assessn complications. Amniotic flu

survival.^{5,8} The major risks associated with expectant management include chorioamnionitis, endometritis, maternal and fetal sepsis, stillbirth, cord prolapse, fetal distress, and fetal positional deformities.^{1-4, 9} Considerations in the management of the patient with preterm premature rupture of membranes should include a careful assessment for development of these potential

Amniotic fluid assessments in patients with preterm premature rupture of membranes have been advocated in delineating patients at increased risk for infection, shortened latency periods, and increased perinatal mortality. ¹⁰⁻¹³ Amniotic fluid assessments have also been helpful in estimating the potential response to tocolysis. ¹⁴ The nonstress test (NST) in preterm premature

to this problem. A prolonged latency period (>48

hours) has been correlated with improved perinatal

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Reprint requests: James A. Harding, MD, Department of Maternal-Fetal Medicine, University of California, Irvine Medical Center, 101 The City Dr., Orange, CA 92668. 6/6/31424 rupture of membranes has been used to identify labor, variable decelerations,9 intrauterine infection, and possible neonatal sepsis.11, 15 Decreased amniotic fluid volumes have been correlated with an increased incidence of cord compressions and fetal heart rate decelerations.9, 16, 17

The interrelation between the amniotic fluid volume and NST findings during the latency period of patients with preterm premature rupture of membranes has not been previously reported. We investigated daily changes in the amniotic fluid volume and correlated NST findings with these changes.

Material and methods

Patients with preterm premature rupture of membranes were prospectively collected from June 1989 until July 1990 from the patient population at Women's Hospital, Long Beach Memorial Medical Center, Long Beach, California. The inclusion criteria were singleton pregnancies with estimated gestational ages ranging between 23 and 34 weeks at the time of premature rupture of membranes. In addition, study patients had to have a minimum of 48 hours of latency, to allow for two consecutive daily amniotic fluid index measurements. Patients with chronic hypertension, systemic lupus erythematosus, diabetes, genital bleeding, multiple gestation, evidence of intrauterine growth retardation, or major fetal congenital anomalies were excluded.

Rupture of membranes was confirmed on admission by sterile speculum examinations with pooled fluid, ferning, and Nitrazine paper determinations. All patients were initially evaluated for evidence of labor, chorioamnionitis, and fetal distress, and, if appropriate, an assessment of fetal lung maturity was obtained. Estimated gestational ages were obtained by best obstetric dates, confirmed by an admission ultrasonographic examination, and stratified for each evaluation. Continuous fetal monitoring was performed for the first 24 hours after premature rupture of membranes and extended if any evidence of active labor or fetal compromise occurred. If undelivered, the patients were placed in the antepartum suite for in-hospital bed rest with vital sign evaluations every 4 hours and minimal activity limited to bathroom privileges. Daily 60-minute NSTs were performed on all patients and evaluated for reactivity, fetal decelerations, persistent fetal tachycardia, and uterine activity.

Amniotic fluid assessments were performed at similar times daily, by means of the amniotic fluid index with the four-quadrant technique described by Phelan et al.18 Daily amniotic fluid index results were blinded to the managing physicians. Patients were delivered for evidence of chorioamnionitis, fetal distress, spontaneous labor, or fetal lung maturity. The relative change in the amniotic fluid index from one day to the next was calculated by the difference between the previous day's index and the present day's index, divided by the previous day's index and then multiplied by 100. The overall average daily amniotic fluid index and relative daily change were the two primary outcome variables assessed in this study. These two outcome variables were then correlated to parity, gestational age, fetal position, uterine activity, fetal heart rate reactivity, and the presence of variable decelerations.

The NST evaluations for clinical management were performed daily and evaluated by the attending physician. For purposes of this report, all NSTs were reviewed without knowledge of the amniotic fluid index by a single interpreter. Fetal heart reactivity was defined as two or more fetal heart rate accelerations of ≥15 beats/min in amplitude and ≥15 seconds' duration during a 20-minute period. A variable deceleration was defined as a 30 beats/min decrease below the baseline heart rate for ≥15 seconds. Uterine activity was assessed during the 1-hour segment of the NST and was defined as the number of uterine contractions not associated with active labor identified during that hour.

Interobserver error was obtained by two observers comparing blinded amniotic fluid index examination within 1 hour of each other. Intraobserver error was calculated by the same observer repeating the amniotic fluid index examination within 1 hour of the previous examination.

Statistical analysis was performed incorporating χ^2 with Yates' correction, Fisher's exact test, and linear regression. All tests were against a two-sided alternative hypothesis, and a p < 0.05 was considered statistically significant. Results are reported in mean values ± 1 SD in the tables and the text.

Results

A total of 50 patients were enrolled in the study, and 422 individual examinations were performed. The average number of evaluations per patient was 10.3 (range 2 to 38). The study group analysis revealed a mean maternal age of 28.6 ± 4.3 years; 15 (30%) were nulliparous. The average gestational age at the time of preterm premature rupture of membranes was 29.7 ± 2.7 weeks (range 23.9 to 35.1 weeks) with a delivery age average of 30.7 ± 2.6 weeks (range 24.3 to 36.0 weeks). The overall average amniotic fluid index was 5.9 ± 2.1 cm (range 1.3 to 16.0 cm), and the average daily variation was $7.1\% \pm 45.1\%$ per day (range -74.0% to 205.0% per day).

Fig. 1 represents the scattergram comparing the daily amniotic fluid index evaluation with the gestational age at the time of the examination with the mean amniotic fluid index ± 2 SD delineated. The daily amniotic fluid index increases statistically with increasing gestational age, as seen in Table I, using linear regression analysis.

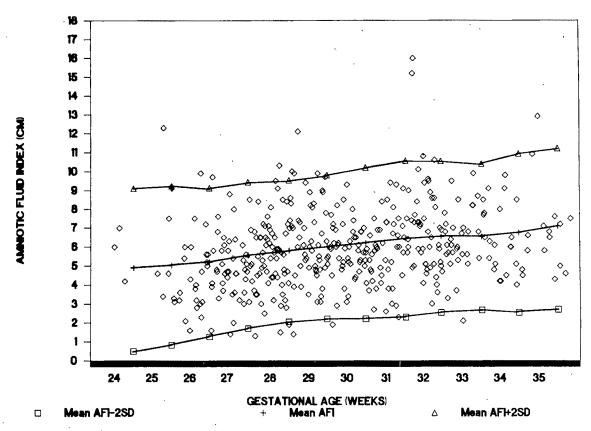


Fig. 1. Amniotic fluid index (AFI) in centimeters recorded in patients with premature rupture of membranes relative to gestational age at measurement in weeks. Mean anniotic fluid index and ± 2 SD curves are displayed.

Table I. Overall daily amniotic fluid index and variability in patient with preterm premature rupture of membranes in relation to gestational age, demonstrating statistically significant linear relationship (r = 0.27)

Gestational age (wk)	No. of evaluations	Daily amniotic fluid index (cm, mean ± 1 SD)	Change in amniotic fluid index (%/day, mean ± 1 SD)
23-24	4	5.5 ± 1.1	27.6 ± 53.1
25-26	47	4.9 ± 2.1	13.8 ± 65.5
27-28	119	5.6 ± 2.0	8.4 ± 49.4
29-30	104	5.8 ± 1.6	4.3 ± 31.8
31-32	. 96	6.6 ± 2.3	6.4 ± 44.2
33-34	40	6.5 ± 1.9	2.1 ± 32.8
35-36	12	7.1 ± 2.3	6.5 ± 28.2
p Value		< 0.005	NS

In comparison though, the rate of change in amniotic fluid index from one day to the next, also depicted in Table I, does not vary statistically as the gestational age increases.

Table II outlines the overall average amniotic fluid index and its day-to-day change in relation to parity, fetal position, and uterine activity. The amniotic fluid index was statistically lower in the nulliparous patient but was not significantly affected by fetal position or

uterine activity. The day-to-day variation was not significantly altered by parity, position, or uterine activity (Table II).

Nulliparous patients also had higher rates of variable decelerations compared with multiparous patients (40.0% vs 27.6%, respectively; p < 0.02), which appears to be unrelated to gestational age (29.8 \pm 2.7 vs 29.9 \pm 2.5 weeks, respectively; p not significant).

Table III reviews the incidence of variable deceler-

Table II. Overall daily amniotic fluid index and variability in patient with preterm premature rupture of membranes in relation to parity, fetal position, and number of uterine contractions per hour (uterine contraction statistical analysis by linear regression with respective r values documented)

Parameter	No. of evaluations	Daily amniotic fluid . index (cm, mean ± 1 SD)	Change in amniotic fluid index (%/day, mean ± 1 SD)
Parity			
0	125	5.4 ± 2.1	4.8 ± 48.9
≥l	297	6.2 ± 2.0	8.0 ± 43.4
p Value	→	< 0.001	NS
Fetal position			
Vertex	358	5.9 ± 2.1	6.8 ± 44.4
Breech on transverse lie	64	5.9 ± 2.0	8.8 ± 49.2
p Value		NS	NS
Uterine contractions/hour			
0	207	5.8 ± 2.0	8.3 ± 48.3
1-2	95	6.2 ± 2.3	9.6 ± 42.8
3-5	69	6.4 ± 2.2	6.0 ± 39.0
6-9	32	5.8 ± 2.0	2.2 ± 46.5
≥10	19	5.3 ± 2.2	-6.4 ± 38.0
p Value	_	NS (r = 0.07)	NS (r = 0.08)

ations identified on the NST with a correlated amniotic fluid index measurement. The incidence of variable decelerations in the extremely low examination (0 to 1.9 cm) was 50% in comparison with an incidence of 0% in an amniotic fluid index \geq 10 cm (p < 0.01) by linear regression analysis.

Table IV reviews the relationship of the amniotic fluid index and its day-to-day changes with the corresponding alterations in the NST observations. Nonreactive NSTs were associated with a statistically lower amniotic fluid index than were reactive NSTs $(4.6 \pm 2.2 \text{ vs } 6.0 \pm 2.0 \text{ cm}, \text{ respectively; } p < 0.001).$ The variance of the day-to-day changes was high, ranging from 43.5% to 60.5% per day. Variable decelerations were associated with a statistically lower amniotic fluid index $(5.3 \pm 1.7 \text{ cm})$ versus no decelerations $(6.2 \pm 2.2 \text{ cm}), p < 0.001$. Gestational age was statistically lower in the patient evaluations associated with variable decelerations, nonreactive NSTs, and lower daily amniotic fluid index. The variance again was high in the day-to-day change in amniotic fluid index in association with the presence of variable decelerations, so no statistical correlation could be identified.

Tables V and VI tabulate further the changes in the NST outcome variables from one day to the next and lists the corresponding amniotic fluid index values. Table V indicates that the development of variable decelerations is associated with an overall lower amniotic fluid index, although the variance is high in the dayto-day change (38.8% to 43.7% per day) leading to statistical insignificance. Gestational age appeared to have no significant association with the development of variable decelerations nor with the changes in reactivity

Table III. Overall daily amniotic fluid index in patient with preterm premature rupture of membranes and corresponding relationship to presence of variable decelerations

Daily amniotic fluid index (cm)	No. of evaluations	Variable decelerations present (%)
0-1.9	8	50.0
2-3.9	57	38.6
4-5.9	160	36.8
6-9.9	185	25.4
≥10	12	0.0
p Value	_	< 0.01

 $\chi^2 = 13.04$, degrees of freedom = 4.

as seen in Tables V and VI, respectively. Table VI reveals a statistically lower amniotic fluid index found in the development of a nonreactive NST, although the change from the previous day is not statistically significant. The daily amniotic fluid index is lower in the nonreactive NST, as noted in Table IV, and is seen in Table VI as well, although the change from a nonreactive to a reactive NST does not have a statistically greater amniotic fluid index.

A total of 13 patients were randomly selected for development of the interobserver and intraobserver error for the amniotic fluid index examination. Intraobserver error was found to be 1.8 ± 1.6 cm or a relative variation of $45.7\% \pm 60.8\%$ (r = 0.64), with a mean time difference between examinations of 25.6 ± 22.2 minutes. The interobserver error was 1.7 ± 1.3 cm or a relative variation of $31.3\% \pm 18.9\%$ (r = 0.65), with

Table IV. NST results (presence of reactivity or variable decelerations) in patient with preterm premature rupture of membranes as compared with gestational age, fetal position, daily amniotic fluid index, and change in amniotic fluid index from the previous day

NST results	No. of evaluations	Gestational age (wk, mean \pm 1 SD)	Vertex present (%)	Daily amniotic fluid index (cm, mean ± 1 SD)	Change in amniotic fluid index (%/day, mean ± 1 SD)
Reactive Nonreactive p Value	393 29	$\begin{array}{c} 30.0 \pm 2.6 \\ 28.6 \pm 2.1 \\ < 0.01 \end{array}$	84.2 93.1 NS	6.0 ± 2.0 4.6 ± 2.2 <0.001	7.5 ± 43.5 0.3 ± 60.5 NS
No decelerations Decelerations p Value	290 132 —	30.2 ± 2.5 29.2 ± 2.5 <0.01	83.8 87.1 NS	6.2 ± 2.2 5.3 ± 1.7 < 0.001	8.7 ± 45.3 3.6 ± 44.6 NS

Table V. Changes from previous-day NST in the identification of fetal heart rate decelerations and corresponding relation to gestational age, amniotic fluid index, and change from previous day amniotic fluid index

Change in NST results	No. of evaluations	Gestational age $(wk, mean \pm 1 SD)$	Daily amniotic fluid index (cm, mean ± 1 SD)	Change in amniotic fluid index (%/day, mean ± 1 SD)
No deceleration to no deceleration	236	30.3 ± 2.5	6.3 ± 2.2	6.6 ± 43.7
No deceleration to deceleration	57,	29.7 ± 2.5	5.4 ± 1.5	0.6 ± 38.8
p Value	-	NS	< 0.01	NS
Deceleration to deceleration	75	28.8 ± 2.3	5.2 ± 1.8	5.7 ± 48.0
Deceleration to no deceleration	54	29.6 ± 2.5	6.0 ± 2.0	17.4 ± 50.2
p Value	attonados	NS	< 0.02	· NS

NS, Not significant.

Table VI. Alterations in day-to-day reactivity of NST relative to gestational age, amniotic fluid index, percent change in amniotic fluid index, and percentage with fetal heart rate decelerations

Change in NST results	No. of evaluations	Gestational age (wk, mean ± 1 SD)	Daily amniotic fluid index (cm, mean ± 1 SD)	Change in amniotic fluid index (%/day, mean ± 1 SD)	With decelerations (%)
Reactive to reactive Reactive to nonreactive p Value	377 18	30.0 ± 2.6 29.2 ± 2.2 NS	$6.1 \pm 2.0 \\ 4.9 \pm 2.3 \\ < 0.02$	7.0 ± 42.9 -10.0 ± 36.8 NS	29.7 55.5 <0.04
Nonreactive to nonreactive Nonreactive to reactive p Value	11 16	27.7 ± 1.5 28.3 ± 1.6 NS	4.0 ± 1.9 5.4 ± 1.9 NS	17.5 ± 83.5 20.9 ± 55.9 NS	36.4 37.5 NS

NS, Not significant.

a mean time between the two examinations of 10.5 ± 6.9 minutes.

Comment

Amniotic fluid regulation in the patient with intact membranes is closely governed and remains fairly static on a day-to-day basis. ¹⁹ Amniotic fluid volumes, when measured with the amniotic fluid index, vary considerably on a day-to-day basis (7.1% \pm 45.1% per day) during the latency period in the patient with preterm premature rupture of membranes. Percent changes on

a daily basis indicate the fluid may decrease by as much as 75% and increase by up to 200% from one day to the next. The daily change in amniotic fluid index is of such magnitude that no individual variable seems to have a statistically significant effect on this change.

The amniotic fluid index variation with gestational age in patients with intact membranes has been well defined.^{18, 20} The amniotic fluid index has a gradual increase in value with advancing gestational age until approximately 24 to 26 weeks, at which point it levels

off until 34 weeks and then begins to decline. In the patient with preterm premature rupture of membranes, in contrast, it progressively increases with advancing gestational age, even past 32 weeks in our study population. The mean daily amniotic fluid index in these patients is <1st percentile by the standard curves generated for intact membranes.20 Nonreactive NST and presence of variable decelerations are noted in the lower gestational ages. It is uncertain if the gestational age alone accounts for these findings or whether they are caused by the lower amniotic fluid index values. One should conclude, though, that in the earlier gestations the higher the risk for variable decelerations, nonreactive NSTs and lower amniotic fluid index values, and therefore the higher risk for possible fetal compromise.

Uterine activity and fetal position surprisingly had little overall effect on the amniotic fluid index in the patient with preterm premature rupture of membranes in the nonlaboring state. The data suggest an overall loss of fluid from the previous day, when there were more than 10 contractions in the hour identified (-6.4% per day), but this was not statistically significant because of the broad variance. The uterine activity noted on the NSTs was generally asymptomatic and of little magnitude compared with that in the laboring patient.

The development of variable decelerations was noted with a lower overall amniotic fluid index, which correlates well with the work by Gabbe et al.,16 indicating an increase in variables when the amniotic fluid was removed from the chronic fetal rhesus monkey preparations. Patients with preterm premature rupture of membranes have a higher incidence of variable decelerations in labor and a subsequent higher incidence of fetal distress, possibly related to the decrease in amniotic fluid volume.9 At the same gestational age, nulliparous patients maintain statistically significant lower amniotic fluid indexes than multiparous patients. The explanation for this is uncertain, but with the lower amniotic fluid volumes the nulliparous patient tends to have a higher incidence of variable decelerations. The presence of nonreactivity in the NST is also correlated with a statistically lower amniotic fluid index (6.0 cm in the reactive NST vs 4.6 cm in the nonreactive NST). The development of nonreactivity or significant variable decelerations correlates with possible fetal compromise and infection.9, 11, 15

The major difficulty in using the amniotic fluid index measurement to identify the potentially compromised fetus is the innate precision error of the test. For example, the difference between the amniotic fluid indexes associated with the development of variable decelerations or nonreactivity is 0.9 cm and 1.2 cm, respectively. The interobserver error was 1.7 ± 1.3 cm in our population and is reported to be 0.97 cm²⁰ to 2.0 cm in intact membranes.21 The interobserver errors are statistically greater than the amniotic fluid index differences correlated with development of abnormal NST findings, indicating that the decrease in the amniotic fluid index may not be measured by the actual examination but may be suggested by the findings on the NST. Therefore, by measuring the amniotic fluid index only, a significant change in amniotic fluid index may not be appreciated because of the lack of precision of the test in the patient with preterm premature rupture of membranes.

In conclusion, the amniotic fluid index varies widely on a day-to-day basis in the latency period after preterm premature rupture of membranes. No one specific identifiable factor affects its day-to-day alteration. The overall index is lower in the nulliparous patient and in the earlier gestation. A lower amniotic fluid index is associated with an increased incidence of development of variable decelerations and nonreactivity in the NST. The average differences in the index associated with the development of these factors is beyond the precision of the examination. The NST evaluation in the prolonged latency period after preterm premature rupture of membranes provides better potential insight into the clinically reduced amniotic fluid volumes than the measured amniotic fluid index in the patient. The value remaining in amniotic fluid index assessment in the patient with preterm premature rupture of membranes may be in the initial examination where the amniotic fluid volume appears to be predictive of infection, duration of latency period, and response to tocolytic therapy. 10-12, 14

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Variable decelerations in reactive nonstress tests with decreased amniotic fluid index predict fetal compromise

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A total of 3158 patients at ≥34 weeks' gestation undergoing nonstress tests and amniotic fluid index determinations were divided into six groups according to the amniotic fluid index and the nature of the decelerations. Fetuses with antepartum decelerations had statistically significantly increased incidences of intrapartum decelerations and operative deliveries because of intrapartum "distress," regardless of the amniotic fluid index. They also had significantly increased rates of neonatal acidosis and low Apgar scores when there were "severe" decelerations and an amniotic fluid index <5 in the antepartum period. Thus spontaneous decelerations in reactive nonstress tests with an amniotic fluid index <5 may predict fetal compromise. (AM J OBSTET GYNECOL 1991;165:1094-8.)

Key words: Antepartum decelerations, oligohydramnios, fetal compromise

The reliability of antepartum fetal surveillance techniques to determine fetal well-being continues to be debated. There are conflicting reports in the literature

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regarding the association of variable decelerations during nonstress tests (NSTs) and fetal compromise. 1-6 Sarno et al.7 have suggested that the amniotic fluid index is a useful adjunct to antepartum surveillance. They found that an amniotic fluid index ≤ 5 in the early intrapartum period was a risk factor for abnormal intrapartum fetal heart rate patterns.

The current study was conducted to assess the intrapartum and neonatal implications of variable decelerations occurring during reactive NSTs in patients with antepartum oligohydramnios.

Table I. Frequency of intrapartum decelerations

,		var	partum iable rations	Intrapar deceler	
	n	No.	%	No.	%
Group 1: Reactive NST + amniotic fluid index >5	2132	490	23	235	11
Group 2: Reactive NST + amniotic fluid index ≤5	382	99	26	61	16
Group 3: Mild variable decelaration + amniotic fluid index >5	181	45	25	24	13
Group 4: Mild variable decelerations + amniotic fluid index ≤5	109	58	53*	23	21
Group 5: Severe variable decelerations + amniotic fluid index >5	236	142	60*	45	19
Group 6: Severe variable decelerations + amniotic fluid index ≤5	118	86	73†	31	26

^{*}p < 0.05.

Material and methods

The study population consisted of patients with singleton, nonanomalous pregnancies ≥34 gestational weeks with intact membranes undergoing NSTs, amniotic fluid index assessment, and subsequent delivery at our institution from July 1987 to July 1990. Patients had weekly NSTs while lying in the lateral or semirecumbent position. The indications included diabetes, hypertension, suspected growth abnormalities, preeclampsia, underlying medical conditions, and previous poor obstetric history. Excluded were studies from 274 (7.9%) patients that showed uniform late decelerations, more than six contractions per hour, decelerations exceeding 1 minute's duration, absence of reactivity after 2 hours, or results that were uninterpretable because of technical reasons.

Reactive studies were defined by the presence of at least two 15-beat accelerations of at least 15 seconds' duration during a 20-minute period. For the purposes of this study, the decelerations were classified as mild or severe. Mild spontaneous variable decelerations were those lasting ≤ 15 seconds with a decline ≤ 20 beats/min below baseline. Severe spontaneous variable decelerations were those lasting >15 seconds with a decline >20beats/min below baseline.

Amniotic fluid index estimates were made with the four-quadrant technique previously described.8,9 A linear-array, real-time B scan with electronic calipers was used with the patients in the supine position. Landmarks for the four quadrants of the maternal abdomen included the umbilicus, which divides it into upper and lower halves, and the linea nigra, which divides it into right and left halves. The linear transducer was placed along the mother's longitudinal axis and held parallel to the floor for all measurements. The maximum vertical diameter of the largest fluid pocket was measured

in centimeters in each quadrant. Brief appearances of cord or an extremity were ignored, but aggregation of either one resulting in exclusion of fluid was excluded. The measurements obtained from each quadrant were summed to form the amniotic fluid index. An amniotic fluid index >5.0 was considered to reflect adequate fluid.8

When more than one type of deceleration appeared on the NST, the patients were grouped according to the most severe NST variable. These were matched to the lowest amniotic fluid index noted at any test performed within 10 days of delivery. Pregnancy outcome was assessed with respect to the following: (1) incidence and type of intrapartum decelerations, (2) fetal distress that required operative delivery (forceps or cesarean section), (3) Apgar scores <7 at 5 minutes, (4) neonatal acidosis (pH <7.20), (5) occurrence of terminal meconium, and (6) incidence of nuchal cords.

Fetal distress was defined as fetal bradycardia or fetal acidosis occurring at any time while the woman was in labor. At no time were the findings of the antepartum assessments (NST and amniotic fluid index) used to diagnose fetal distress. Fetal bradycardia was defined as a fetal heart rate <90 beats/min or a decline in rate of 40 beats/min below the baseline, lasting for >3 minutes. Fetal acidosis was defined as a scalp pH <7.20.

Sensitivity, specificity, and predictive values for fetal distress necessitating cesarean delivery were calculated for each variable. Statistical analysis was carried out with Fisher's exact test or χ^2 analysis, and significance was considered as p < 0.05.

Results

There were 3158 patients in the study who underwent NST and amniotic fluid index determination. The mean \pm SD for maternal age was 25.0 \pm 4.3 years

 $[\]uparrow p < 0.001$.

Table II.	Incidence	of operati	ve delivery	because o	f fetal	distress
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		Forceps	delivery	Cesarear	ean delivery	
	n	No.	%	No.	%	
Group 1: Reactive NST + amniotic fluid index >5	2132	128	6	149	7	
Group 2: Reactive NST + amniotic fluid index ≤5	382	34	9	42	11	
Group 3: Mild variable decelerations + amniotic fluid index >5	181	16	9	. 22	12	
Group 4: Mild variable decelerations + amniotic fluid index ≤5	109	16	15	36 ,	33*	
Group 5: Severe variable decelerations + amniotic fluid index >5	231	25	11	95	41*	
Group 6: Severe variable decelations + amniotic fluid index ≤5	118	15	13	89	75†	

^{*}p < 0.05.

(range, 16 to 38 years). The estimated gestational age (mean \pm SD) was 36.3 \pm 2.0 weeks (range, 34 to 43 weeks). The medical and high-risk conditions were found to be equally distributed and not significantly different among the groups. There were 2132 patients in group 1, where the NST was reactive and the amniotic fluid index was >5. In group 2 there were 382 patients who had reactive NSTs but an amniotic fluid index ≤5. The 181 patients with mild spontaneous variable decelerations on NST and an amniotic fluid index >5 made up group 3. Group 4 had 109 patients; they showed decelerations similar to those in group 3 but had an amniotic fluid index ≤5. The 236 women who had severe spontaneous variable decelerations on NST and an amniotic fluid index >5 made up group 5. There were 118 women in group 6, in whom the decelerations were similar to those in group 5 but the amniotic fluid index was ≤5.

There was a strong association between antepartum decelerations occurring in a repetitive pattern and the finding of similar repetitive decelerations during labor, regardless of amniotic fluid index (Table I). This association achieved statistical significance when either the NST variables were more pronounced or the amniotic fluid index was ≤5. The most frequent occurrence of this association was seen when patients had severe variable decelerations on NST and oligohydramnios (amniotic fluid index, ≤5). Table II describes the occurrence of operative delivery (forceps or cesarean section) for the patients in the study. Once again, patients in groups 4, 5, and 6 had much higher rates of operative delivery because of fetal distress. The most frequent association was in group 6, in which 88% (104/118) had operative delivery.

Neonatal outcomes of these patients are shown in Table III. The birth weight percentiles were equally distributed and were not significantly different in the

six groups. The women in group 6 had a statistically significantly increased incidence of neonatal acidosis (37/118, 31%) and low 5-minute Apgar scores (15/118, 13%). There was a 2% incidence of nuchal cords and a 7% incidence of terminal meconium in the patients in group 1 (i.e., reactive NST and amniotic fluid index >5). In groups 2 through 6 the ranges of incidence of these events were 2% to 5% and 9% to 13%. These findings were similar to those of other investigators. 6, 10, 11 and were not statistically significant.

The sensitivity, specificity, and predictive values of mild and severe variable decelerations on NST and amniotic fluid index ≤5 because of subsequent fetal distress necessitating operative delivery and because of subsequent neonatal acidosis are shown in Table IV.

Comment

The idea of an antepartum risk scoring test is an attractive one because it might help to identify the 10% to 20% of low-risk patients who subsequently have intrapartum complications. 12, 13 Such a risk scoring system may allow patients to be triaged into high-risk or lowrisk status. Once a patient is considered high risk, her physician can be alerted to an increased risk of fetal intolerance to labor.

Previous authors have evaluated other diagnostic modalities in an attempt to predict intrapartum and neonatal complications. Ingemarsson et al.14 found a 75% risk for fetal distress after a nonreactive NST and acoustic stimulation test. Sarno et al. 15 studied 109 patients during early labor and found that abnormal initial fetal heart rate tracings and low amniotic fluid index results had very low positive predictive values for subsequent fetal distress. Even though these authors did not define fetal distress per se, they did define an abnormal tracing as one with evidence of variable or late decelerations or bradycardia. Additionally, amniotic

 $[\]dagger p < 0.001.$

Table III. Neonatal outcomes (acidosis and Apgar scores)

		acidos	natal is (pH, .20)	5 min Apgar score <7	
	n	No.	%	No.	%
Group 1: Reactive NST + amniotic fluid index >5	2132	107	5	43	2
Group 2: Reactive NST + amniotic fluid index ≤5	382	27	7	12	3
Group 3: Mild variable decelerations + amniotic fluid index >5	181	13	7	5	3
Group 4: Mild variable decelerations + amniotic fluid index ≤5	109	10	9	3	3
Group 5: Severe variable decelerations + amniotic fluid index >5	236	26	11	12	5
Group 6: Severe variable decelerations + amniotic fluid index ≤5	118	37	31*	15	13*

^{*}p < 0.05.

Table IV. Sensitivity, specificity, and predictive values of mild and severe variable decelerations

	Fetal distress necessitating cesarean section					Neonatal acidosis		
	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
"Mild" variable decelerations on NST and amniotic fluid index ≤5	71.5	83.3	23.3	97.9	17.6	86.4	13.2	93.7
"Severe" variable deceler- ations on NST and amniotic fluid index ≤5	84.5	87.2	43.6	91.2	43.8	84.7	18.1	89.9

fluid index results were analyzed in all patients, even though 47.7% had ruptured membranes at the time of entry in the study. On the other hand, in our study all patients had intact membranes at the time of the amniotic fluid index assessment.

The observation that spontaneous variable decelerations (both "mild" and "severe") are predictive of intrapartum variable decelerations has already been shown by others. However, amniotic fluid index assessment was incorporated into the evaluation process in an attempt to identify an additional factor, i.e., oligohydramnios causing cord compression, that may help to predict fetal intolerance to labor. In our patients the existence of oligohydramnios along with variable decelerations seemed to further increase the risks for adverse perinatal outcomes (emergency delivery and neonatal compromise).

The neonatal outcomes in our patients differed from those of Judge et al." who found no increase in neonatal acidosis or low Apgar scores in 693 patients with variable decelerations on NSTs. Some of their patients (12% to 20%) also had decreased amniotic fluid, but

the method and results of the fluid assessments are unavailable. On the other hand, we found a significantly increased incidence of acidotic (pH <7.20) and depressed (5-minute Apgar scores <7) neonates born to mothers who had antepartum severe variable decelerations and an amniotic fluid index \le 5.

The current study was an attempt to use antepartum decelerations and oligohydramnios as a type of screening test to identify subsequent adverse intrapartum and neonatal compromise; therefore we calculated predictive values for these parameters. The positive predictive values were <50% for predicting emergency cesarean sections and <20% for neonatal acidosis. Both parameters showed high specificity (>80%) for adverse outcomes. It seems that, even though these tests can provide information regarding the increased incidence of intrapartum fetal distress or neonatal acidosis and depression, the low positive predictive values suggest that planned cesarean section delivery would not be warranted.

In conclusion, this study suggests that antepartum variable decelerations, especially if associated with decreased amniotic fluid volume, are an ominous finding. They may be useful in predicting subsequent adverse intrapartum and neonatal outcomes.

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Preterm premature rupture of membranes: Detection of infection

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This prospective study was designed to determine the value of a daily modified biophysical profile in detecting infection in patients with preterm premature rupture of the membranes who were managed expectantly. Ninety-nine patients received daily nonstress tests and biophysical profile scores. Results of the last predelivery study were related to subsequent development of amnionitis or fetal sepsis. Infection was present in 16 patients. When the biophysical profile score was 0/8, infection was uniformly present. When fetal breathing was absent (biophysical profile score, ≤4/8) and nonstress test was nonreactive, infection was present in 75% of cases (sensitivity, 75%; specificity, 95%). Because a nonreactive nonstress test could be secondary to prematurity instead of infection, these results were analyzed over time. Those who initially had a reactive nonstress test that subsequently became nonreactive were more likely to be infected. We conclude that a daily biophysical profile score and nonstress test can detect infection and propose delivery of patients with a biophysical profile score of 0/8 and nonreactive nonstress test. Patients with absent fetal breathing and a nonstress test that changes from reactive to nonreactive also should be considered for delivery. Absent fetal breathing with a reactive nonstress test or a consistently nonreactive nonstress test should have further testing to rule out infection. (AM J OBSTET GYNECOL 1991;165:1099-104.)

Key words: Preterm premature rupture of membranes, biophysical profile, chorioamnionitis, fetal sepsis

Preterm premature rupture of membranes, defined as rupture of the membranes before the onset of regular uterine contractions, occurs in approximately 10% of all pregnancies and is associated with significant perinatal mortality and morbidity, primarily from premature delivery, sepsis, and asphyxia. In the preterm gestation, expectant management may minimize the risk of prematurity; however, delay of delivery may increase mortality and morbidity from infection and asphyxia.

If accurate methods were available for early detection of chorioamnionitis or fetal sepsis, outcome in these pregnancies perhaps could be improved. In the past these methods included observation of maternal vital signs, hematologic studies, analysis of amniotic fluid, and electronic fetal monitoring. All of these methods have their own unique deficiencies, and none provide the clinician with an ideal test. Recently, analysis of fetal behavior has been used to evaluate patients with

the presence of infection has been observed.

The purpose of this prospective study was to determine the ability of a daily modified biophysical profile examination to detect chorioamnionitis or fetal sepsis in patients with preterm premature rupture of membranes who were managed expectantly.

preterm premature rupture of membranes. These

studies suggest that the infected fetus, or the fetus ex-

posed to an infected environment, behaves differently

from the noninfected fetus. 4-6 Specifically, a decrease in

the amount of fetal movement and fetal breathing in

Material and methods

Vanderbilt University Medical Center is a level III regional referral center, serving a catchment area of approximately 25,000 deliveries per year. The institution receives approximately 700 inpatient maternal transports per year. The majority of patients in this study were transferred to Vanderbilt from a nearby facility with the diagnosis of preterm premature rupture of membranes.

Inclusion criteria in this study included: (1) documented preterm premature rupture of membranes, (2) singleton pregnancy with no known fetal anomalies, (3) gestational age <37 weeks, and (4) absence of other medical or obstetric complications.

All patients with suspected preterm premature rupture of the membranes were admitted to the labor and delivery suite for evaluation. Rupture of membranes

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Table I. Modified ultrasonographic biophysical profile score

Fetal movements

Score 4 for two episodes of gross fetal movements within 30 min (simultaneous limb and trunk movements count as single episode)

Score 2 for one such episode of fetal movements Score 0 for no gross movements within 30 min Fetal breathing

Score 4 for two episodes of regular rhythmic fetal breathing of >30 sec duration within 30 min Score 2 for one such episode of fetal breathing Score 0 for no breathing within 30 min

The scores for each category are added. The maximum score is 8. Abnormal is defined ≤4. Other variables assessed but not scored include amniotic fluid level, fetal tone, and placental grade.

was documented by vaginal speculum examination with visualized pooled fluid, ferning, and alkaline pH determination. Routine vital signs were obtained, and physical examination was performed. Electronic fetal heart rate and tocodynamometer monitoring was used to detect fetal distress, as well as to document uterine activity. After an observation period of 12 hours, patients without evidence of labor, fetal distress, or infection were transferred to the antepartum unit for expectant management.

A total of 99 patients were entered in the study. Gestational ages were determined by last menstrual period and the results of any first-trimester or early secondtrimester ultrasonographic examinations. A level III ultrasonographic examination also was done at the time of admission in all patients to confirm the estimated gestational age and to rule out anomalies. All patients remained hospitalized for the duration of the study and were managed conservatively with bed rest and bathroom privileges. Cervical cultures were obtained on admission and patients were treated with ampicillin (2 gm in 100 ml normal saline solution infused intravenously every 8 hours) until culture results returned negative. Patients with positive group B streptococcus cultures were treated with the same regimen for 7 days and recultured. Temperature was taken three times a day, and physical examination was performed daily. Bimanual pelvic examinations were avoided unless the patient was believed to be in active labor or a decision to induce labor had been made. A 60-minute nonstress test (NST) was performed in all patients daily until delivery. At our institution, a reactive NST is defined as two or more fetal heart rate accelerations of at least 15 beats/min in amplitude and at least 15 seconds' duration in any 10-minute period. A fetal heart rate tracing that did not meet these criteria was considered nonre-

A modified biophysical profile score was obtained

every morning after breakfast in all patients by means of a real-time ultrasonographic method equipped with a 3.5 MHz curvilinear-array transducer. The method used has been previously described and is reviewed here only briefly.7 Real-time scanning consisted of a 30minute observation period during which fetal breathing movements and fetal body movements were observed. Each of the two biophysical variables was scored as 0, 2, or 4, according to the criteria in Table I.7 The modified biophysical profile was repeated every 24 hours until delivery. An abnormal profile, defined as a score of ≤4/8, was repeated twice with at least a 1-hour interval to reduce the risk of a low score being assigned to a normal fetus that is exhibiting normal periodicity of activity. The biophysical profile was not repeated once the diagnosis of amnionitis or labor was made. Results of the biophysical profile were not used in management decisions for any of the patients.

Indications for delivery in this patient population included labor, clinical diagnosis of chorioamnionitis, persistent spontaneous variable decelerations (moderate to severe)8 on electronic fetal heart rate monitoring, or mature fetal lung indices of amniotic fluid obtained by amniocentesis (lecithin/sphingomyelin ratio ≥3.5, phosphatidylglycerol present) (unpublished data, Vanderbilt University Hospital reference laboratory). The diagnosis of clinical chorioamnionitis was made in the presence of two or more of the following criteria: maternal fever >37.8° C, maternal tachycardia (≥120 beats/min), fetal tachycardia (≥160 beats/min), uterine tenderness, foul-smelling amniotic fluid and maternal leukocytosis (white blood cell count ≥20,000/mm³ with a left shift).9 All patients with clinical chorioamnionitis were given parenteral antibiotics and labor was induced, if they were not in labor. Cesarean section was performed for the usual obstetric indications.

On admission of all infants to the neonatal intensive care unit, blood, urine, and cerebrospinal fluid cultures were obtained. Chest x-ray films were obtained when indicated. Prophylactic antibiotics were administered and discontinued only when cultures were negative. The diagnosis of infection was made if blood, urine, or cerebrospinal fluid cultures were positive or if chest x-ray findings were consistent with pneumonia. Once neonatal sepsis was diagnosed, antibiotics were continued for 7 to 10 days.

Data collected after delivery included 1- and 5-minute Apgar scores, birth weight, umbilical artery cord blood pH (only if 1-minute Apgar score ≤7), route of delivery, placental cultures and pathologic evaluation, and results of neonatal septic workup. The results of the last modified biophysical study and NST before delivery were compared with the outcome of pregnancy in regard to the development of infection (clinical chorioamnionitis or neonatal sepsis). The association of

Table II. Biophysical variables of 16 infected cases

Case No.	NST	Fetal breathing	Fetal movement	Diagnosis
1	Nonreactive	0	0	Amnionitis, neonatal sepsis
2	Nonreactive	0	0	Amnionitis
3	Reactive	0	4	Amnionitis
4	Reactive	4	4	Amnionitis
5	Nonreactive	0	2	Amnionitis
6	Nonreactive	Ó	4	Amnionitis
7	Nonreactive	0	4	Amnionitis
8	Nonreactive	0	0	Neonatal sepsis
9	Nonreactive	0	0	Neonatal sepsis
10	Nonreactive	0	4	Amnionitis 1
11	Nonreactive	0	4	Amnionitis
12	Nonreactive	0	4	Amnionitis
13	Nonreactive	0	4	Amnionitis, neonatal sepsis
14	Nonreactive	0	2	Amnionitis
15	Reactive	0	4	Amnionitis
16	Reactive	0	4	Amnionitis

Table III. Demographic characteristics according to infectious outcome

Variable	Infection present $(n = 16)$	Infection $absent$ $(n = 83)$	p Value
Maternal age (yr)	25.1 (7.3)	25.7 (5.9)	NS
Parity	•		
Nulliparous	8	31	NS
Multiparous	8	52	NS
Gestational age (wk)			
Admission	27.4 (3.2)	30.9 (2.9)	< 0.001
Delivery	28.4 (3.2)	32 (2.8)	< 0.001
Birth weight (gm)	1285 (536)	1835 (60Ó)	< 0.002
Apgar scores ≤7	,	,	
1 min	15	41	< 0.002
5 min	6	. 22	NS
Cord pH	7.34 (0.13)	7.30 (0.10)	NS
1	(n = 12)	(n = 35)	
Mean prolongation of pregnancy (days)	8.62 (5.4)	8.67 (6.7)	NS

Values in parentheses are ±2 SD from mean. NS, Not significant.

each biophysical variable and combinations of variables with the outcome was determined. Comparisons were made by using a two-sample t test or Fisher's exact test where appropriate.

Results

We performed 894 examinations among the 99 patients. The mean number of examinations per patient was 9 (range, 1 to 34). The mean gestational age on admission was 30.3 weeks (range, 24 to 35), the mean gestational age at delivery was 31.5 weeks (range, 24 to 37), and the mean prolongation of pregnancy was 8.7 days (range, 1 to 34 days). Of the 99 patients, chorioamnionitis or neonatal sepsis developed in 16 (16.1%). Fourteen of the 16 patients were diagnosed with amnionitis and four were diagnosed with neonatal sepsis. Two patients had both chorioamnionitis and neonatal sepsis (see diagnosis column in Table II). All 14 cases with amnionitis were confirmed by placental histologic examination.10 In the two cases of neonatal sepsis without clinical chorioamnionitis the placental histologic examination revealed chorioamnionitis in only one case.

Table III illustrates the demographic characteristics of the patients according to infectious outcome. There were no differences among the two groups with respect to maternal age, parity, 5-minute Apgar score, cord pH, or mean prolongation of pregnancy. The infected group had significantly lower mean gestational age on admission and at delivery, a lower birth weight (p < 0.01), and a higher incidence of low 1-minute Apgar scores (p < 0.01). Of the 16 infected cases delivery was performed in nine because of diagnosed antepartum amnionitis, in five because of simultaneous onset of labor and amnionitis, and in two because of spontaneous onset of labor alone. Of the 83 noninfected cases delivery was performed in seven because of persistent spontaneous variable decelerations, in two because of persistent vaginal bleeding, in six for mature

Table IV. Biophysical variables or combinations of variables and infectious outcome

		Infection	is outcome
Biophysical variable	Total No. of patients $(N = 99)$	Infected (n = 16)	Noninfected $(n = 83)$
Nonreactive NST	40	12	28
Absent fetal breathing	27	15	12
Absent fetal movement	4	4	0
Nonreactive NST and ab- sent fetal movement	4	4	0
Nonreactive NST and absent fetal breathing	16	12	4
Absent fetal movement and absent fetal breathing	. 4	4	0
Nonreactive NST and ab- sent fetal movement and fetal breathing	4	4	0
Biophysical profile ≤4/8	27	15	12
Nonreactive NST and bio- physical profile score ≤4/8	16	12 .	4

Table V. Ability of each variable to predict infection

Biophysical variable	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Nonreactive NST	75.0	66.3	30.0	93.2	67.7
Absent fetal breathing	93.8	86.7	55.6	98.6	86.7
Absent fetal movement	25.0	100.0	100.0	87.4	87.9
Nonreactive NST and absent fetal breathing	75.0	95.2	75.0	95.2	91.9
Nonreactive NST and absent fetal movement	25.0	100.0	100.0	87.4	87.9
Absent fetal movement and fetal breathing	25.0	100.0	100.0	87.4	87.9
Biophysical profile ≤4/8	93.8	85.5	55.6	98.6	86.9
Nonreactive NST score and biophysical profile score ≤4/8	75.0	95.2	75.0	95.2	91.9

Prevalence = 0.16.

fetal lung indices, and in 68 because of spontaneous labor. The cesarean section rate for the total population was 26% (26/99). Surgical indications included malpresentation (14/26), fetal distress (5/26), repeat cesarean section (5/26), and prolapsed cord (2/26).

There were 20 positive cervical culture results, three in the infected group (19%) and 17 in the noninfected group (20%). The pathogens recovered in the infected group included group B streptococcus (n = 2) and Candida albicans (n = 1). In the noninfected group the pathogens included group B streptococcus (n = 9), Staphylococcus aureus (n = 3), C. albicans (n = 3), gonococcus (n = 1), and group A streptococcus (n = 1). There was no significant difference between groups as to type and duration of antibiotic use.

The relationship between biophysical variables and infectious outcome is illustrated in Table IV. In 68 instances where the biophysical profile was repeated because of an initial abnormal score earlier in the day, 27 remained abnormal and 41 normalized. With rare ex-

ceptions, when the biophysical profile score was 4/8, absent fetal breathing accounted for the low score. There were no patients with absent fetal movement only. The calculated sensitivity, specificity, and positive and negative predictive values of each variable are listed in Table V. Illustrated in Table II are the individual score components of each case that was positive for infection.

With one exception all patients with infection had absent fetal breathing; however, there were 12 patients with absent fetal breathing who did not have infection. When absent fetal breathing was combined with non-reactive NST, there were fewer false-positive results and the test accuracy improved to 92%. The absence of fetal movement was specific for the presence of infection (100%) but had a low sensitivity (25%). When both fetal movement and fetal breathing were absent, all patients were infected and three of four (75%) had fetal sepsis. These neonates all had positive blood cultures. The bacterial organisms isolated were group B

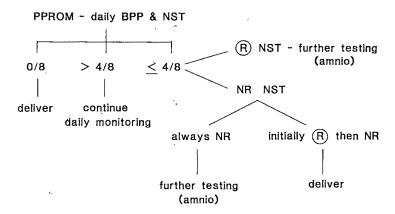


Fig. 1. Suggested protocol for management of patients with preterm premature rupture of membranes (PPROM). BPP, Biophysical profile; R, reactive; NR, nonreactive.

streptococcus (n = 2), Escherichia coli (n = 1), and enterococcus (n = 1).

There were two neonatal deaths and one stillbirth. Patient 8 (Table II) in the infected group was delivered of a 24-week fetus that died at 12 days of age of pulmonary hemorrhage. Patient 2 (Table II) in the infected group was delivered of a stillborn infant at 29 weeks after a cesarean section because of a prolapsed cord, and one patient in the noninfected group was delivered of a 241/2-week fetus that died at 2 months of age as a secondary consequence of renal failure. The perinatal mortality rate was 30:3 per 1000.

The NST résults were further analyzed over time, and these data appear in Table VI. Two of the nine patients who initially had reactive NSTs that then became nonreactive had infection and had absence of fetal breathing. Six of the remaining seven without infection had fetal breathing.

Comment

The expectant management of preterm premature rupture of membranes to gain more time for in utero fetal development may increase the risk of maternal or fetal infection. An accurate test for the early detection of chorioamnionitis or fetal infection is needed. Although many tests have been evaluated, no one test has proved to be ideal.8 The most promising tests have involved the use of ultrasonography to observe fetal biophysical activities with the hypothesis that behavior is different in the presence of infection than in its ab-

Other investigators have examined the relationship between the lack of fetal activity and infection and have found it to be accurate in predicting infectious outcome. 46 Problems and concerns with these studies, however, include the following: retrospective study design, test and outcome variables not independently assessed, use of a broad definition of infectious outcome, and an

Table VI. NST patterns compared with infectious outcome

	Infectious outcome			
NST result	Infected	Not infected		
Always nonreactive $(n = 31)$	10	21		
Initially reactive, becoming nonreactive $(n = 9)$	2*	7†		
Always reactive $(n = 59)$	4	55		

^{*}Absent fetal breathing.

unusually high prevalence of disease.3 This study was designed to avoid these problems by use of a prospective study design in which test results were not used in patient management decisions and by a strict definition of infection as the presence of clinical amnionitis or neonatal sepsis:

The modified biophysical profile method as proposed by Shah et al.7 was chosen because it focuses on investigation of those fetal behaviors that are most likely to be affected by acute compromise of the fetal-maternal unit, namely, fetal movement and fetal breathing. Although the mechanism by which infection alters fetal behavior is not known, our results are consistent with the hypotheses that have been proposed. One such hypothesis is the gradual hypoxia concept,11 which states that in the presence of infection, as a progressive decrease in tissue oxygenation occurs, biophysical variables are lost in the reverse order to which they originally become present, i.e., reactivity of the NST is first to be lost followed by fetal breathing, then fetal movement, then fetal tone, and finally the occurrence of fetal death. Another hypothesis states that, in the presence of bacteria, phospholipase A2 is released, which increases prostaglandin production and may in turn de-

[†]Fetal breathing present in six.

crease fetal breathing. Another factor that may be released in the presence of infection is interleukin-1, which also may alter fetal behavior. It seems reasonable to postulate that as the severity of infection increases, a progressive loss of fetal activity occurs. This is supported by the study results that showed a 100% incidence of infection when the biophysical profile score was 0/8. We would thus recommend delivery for the expectantly managed patient with preterm premature rupture of membranes who has a persistent biophysical profile score of 0/8.

The presence of infection may alter fetal activity in a more subtle manner. Eleven of the 16 infected cases had absent fetal breathing but adequate fetal movement, yielding a biophysical profile score of 4/8. The positive predictive value of this parameter alone was relatively low (57.7%, Table V). To prevent the inappropriate intervention for a noncompromised preterm infant, it would be desirable to use a test with a higher positive predictive value. By combining absent fetal breathing with a nonreactive NST, the positive predictive value increased to 75%.

The accuracy of the NST is gestational age dependent, and it may be difficult to determine if a nonreactive NST is associated with immaturity of the fetal neural cardiac axis or early fetal compromise. The change in fetal behavior states over time may be helpful in evaluating the nonreactive NST in the premature fetus. In this study, if the NST was initially reactive and then became nonreactive, in conjunction with absent fetal breathing, two of the three patients had infection (66%). With loss of fetal heart rate reactivity with fetal breathing present, 6 of 7 (85.7%) did not have infection. Similarly, of those patients with absent fetal breathing and reactive NST, 8 of 11 (73%) did not have infection. However, a reactive NST does not always rule out infection, as 4 of 16 (25%) of the infected cases had a reactive NST.

It is concluded therefore that delivery should be considered for the patient with absent fetal breathing and an NST that has changed from reactive to nonreactive. If there is absent fetal breathing and the NST has always been nonreactive, further testing should be considered to rule out infection, i.e., amniocentesis. Those

patients with absent fetal breathing and reactive NSTs should be monitored closely for developing infection, and amniocentesis may be considered. A suggested protocol for management of preterm premature rupture of membranes is illustrated in Fig. 1.

· In conclusion, daily biophysical profiles and NSTs can detect the presence of infection in patients with preterm premature rupture of membranes. The suggested protocol in Fig. 1 is under investigation to determine if its use improves pregnancy outcome in these high-risk patients.

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Correlation of amniotic fluid glucose concentration and intraamniotic infection in patients with preterm labor or premature rupture of membranes

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Amniotic fluid glucose concentration has previously been suggested as a rapid and sensitive test for diagnosing intraamniotic infection. In this study, 204 patients ≤34 weeks estimated gestational age with preterm labor or premature rupture of membranes underwent amniocentesis to detect subclinical intraamniotic infection. Amniotic fluid was cultured for aerobic and anaerobic bacteria, as well as for *Mycoplasma* species. Amniotic fluid glucose levels were significantly lower in patients with positive amniotic fluid cultures than in patients with negative cultures (median, 10 mg/dl; range, 1 to 62 mg/dl vs median, 31 mg/dl; range, 2 to 126 mg/dl, respectively; *p* < 0.001). In terms of predicting amniotic fluid culture results, an amniotic fluid glucose concentration of ≤16 mg/dl had a sensitivity of 79%, specificity of 94%, positive predictive value of 87%, and negative predictive value of 90%. The determination of amniotic fluid glucose concentration is useful in detecting subclinical intraamniotic infection in patients ≤34 weeks estimated gestational age with preterm labor or premature rupture of membranes. (AM J OBSTET GYNECOL 1991;165:1105-10.)

Key words: Amniotic fluid glucose, intraamniotic infection, prematurity

Subclinical intraamniotic infection has been associated with preterm labor and preterm premature rupture of membranes.1-5 The prompt diagnosis of subclinical intraamniotic infection is important in that it may prevent the maternal and neonatal morbidity associated with clinical chorioamnionitis. 6,7 Amniotic fluid cultures obtained by transabdominal amniocentesis have been used to diagnose early intraamniotic infection in patients with preterm labor or premature rupture of membranes.1-5 The culture results, however, may not be available for several days. Gram stain examination of the amniotic fluid is more rapid but has a low sensitivity, missing up to 55% of cases with intraamniotic infection.8 Other parameters, such as maternal fever, white blood cell count, C-reactive protein, or uterine irritability either occur too late or lack sufficient sensitivity and specificity to be of clinical benefit.9

Determination of amniotic fluid glucose concentration has been proposed as a rapid and sensitive test for the detection of intraamniotic infection in patients with preterm labor.¹⁰ The purpose of this prospective study was to evaluate the usefulness of amniotic fluid glucose concentration in predicting amniotic fluid culture results in patients with preterm labor or premature rupture of membranes.

Material and methods

The study population consisted of patients admitted to the University of Illinois Hospital with the diagnosis of preterm labor or premature rupture of membranes who had an estimated gestational age of ≤34 weeks and no overt clinical evidence of infection. Diabetic patients and patients receiving a β-agonist for tocolysis or betamethasone to enhance fetal lung maturity were included in the study. Patients on antibiotics were excluded from the study. The University of Illinois Hospital primarily serves indigent black and Hispanic patients. The study was approved by the institutional review board of the hospital.

Preterm labor was defined as regular uterine contractions associated with either documented cervical change or an initial cervical examination of ≥2 cm or 80% effacement. Premature rupture of membranes was documented by both a positive Nitrazine paper reaction and ferning test or by visualizing the leakage of amniotic fluid from the cervix.

After informed consent was obtained, transabdominal amniocentesis under ultrasonographic guidance was performed on each patient. The amniotic fluid was immediately placed, without exposure to air, in a transport vial suitable for anaerobic organisms (Port-A-cul, Becton Dickinson, Cockeysville, Md.) and brought to the microbiology laboratory. The amniotic fluid was cultured for aerobic and anaerobic bacteria and for

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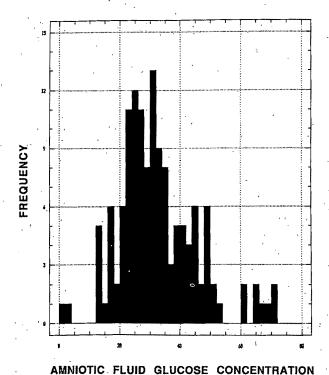


Fig. 1. Amniotic fluid glucose concentration in patients with negative amniotic fluid cultures.

Mycoplasma hominis and Ureaplasma urealyticum. Gram stain for microorganisms was performed, and stained slides were examined by trained microbiology technologists. Intraamniotic infection was defined as a positive amniotic fluid culture.⁵ Clinical chorioamnionitis was defined according to the criteria of Gibbs et al.¹⁰

Amniocentesis is commonly performed at our institution to diagnose subclinical intraamniotic infection in patients \leq 34 weeks' estimated gestational age with preterm labor and premature rupture of membranes. During the study period, a positive Gram stain or a positive culture was an indication for delivery. These patients were started on parenteral antibiotics, and labor was induced or allowed to continue spontaneously. A culture that was positive for U. urealyticum alone was not considered an indication for delivery if there was no clinical evidence of maternal infection and if daily biophysical profiles were reassuring.

Amniotic fluid glucose concentrations were determined within 30 minutes of the amniocentesis. Analysis was performed by the glucose oxidase method with a Beckman glucose analyzer. The results of the amniotic fluid glucose concentrations were not used in patient management. Maternal serum glucose was determined by the same method at the time of amniocentesis.

All infants born prematurely underwent an evaluation for neonatal sepsis, consisting of blood and urine cultures. Cerebrospinal fluid cultures were done at the discretion of the neonatologist. Neonatal sepsis was de-

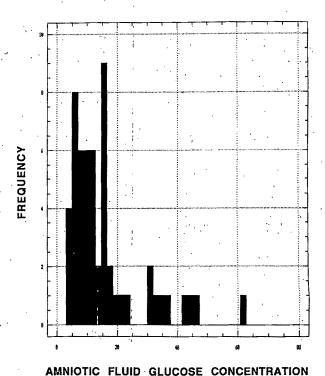


Fig. 2. Amniotic fluid glucose concentration in patients with positive amniotic fluid cultures.

fined as a positive blood, urine, or cerebrospinal fluid culture.

The Mann-Whitney test was used to compare amniotic fluid glucose concentration in patients with positive versus negative amniotic fluid cultures. A receiver operator characteristic curve analysis was performed to determine the relationship between the sensitivity and the false-positive rate for different values of amniotic fluid glucose in the detection of a positive culture. The sensitivity, specificity, and positive and negative predictive values for detecting positive amniotic fluid cultures were determined for amniotic fluid glucose and Gram stain. The sensitivities of amniotic fluid glucose and Gram stain in predicting positive culture results were compared by means of χ^2 analysis.

Results

Two hundred four patients were entered into the study. One hundred thirteen patients (55.4%) had preterm labor, and 91 (44.6%) had premature rupture of membranes. Amniotic fluid cultures were positive in 18 patients (16.0%) with preterm labor and 49 patients (53.8%) with premature rupture of membranes. Overall, the prevalence of positive amniotic fluid cultures in the study population was 32.8%.

Figs. 1 and 2 display amniotic fluid glucose concentrations in those patients with negative and positive cultures, respectively. Amniotic fluid glucose concentrations were significantly lower in patients with positive

amniotic cultures than in patients with negative cultures (median, 10 mg/dl; range, 1 to 62, vs median, 31 mg/dl; range, 2 to 126, respectively; p < 0.001). In analyzing the data from patients with preterm labor and premature rupture of membranes separately, median amniotic fluid glucose concentrations were similar with regard to amniotic fluid culture results. In patients with preterm labor and positive cultures, the median glucose concentration was 8.5 mg/dl, with a range of 2 to 18, versus a median glucose concentration of 31 mg/dl, with a range of 2 to 71, in patients with preterm labor and negative cultures (p < 0.001). In patients with premature rupture of membranes and positive cultures, the median glucose concentration was 11 mg/dl, with a range of 1 to 62, versus a median glucose concentration of 30 mg/dl, with a range of 2 to 126, in patients with premature rupture of membranes and negative cultures (p < 0.001). Maternal serum glucose concentration in patients with positive amniotic fluid cultures did not differ significantly from patients with negative cultures (89.4 mg/dl \pm 22.6 vs 87.8 mg/dl \pm 27.5, respectively).

The sensitivity, specificity, and positive and negative predictive values of amniotic fluid glucose ≤16 mg/dl and Gram stain are displayed in Table I. An amniotic fluid glucose concentration of ≤16 mg/dl was chosen as the cutoff value after analysis of the receiver operator characteristic curve. This was the level at which we felt the tradeoff between the sensitivity and the false-positive rate was most favorable. An amniotic fluid glucose concentration of ≤16 mg/dl was more sensitive than Gram stain in detecting positive amniotic fluid culture results (79.1% vs 29.9%, respectively; p < 0.001).

The eight cases in which amniotic fluid glucose concentration was ≤16 mg/dl and the amniotic fluid cultures were negative are summarized in Table II. Seven patients (87.5%) were delivered within 24 hours of the amniocentesis, with failure of tocolysis in five patients. The remaining two patients, both with a positive Gram stain, had intrapartum chorioamnionitis.

Table III summarizes the 14 cases in which the amniotic fluid glucose concentration was >16 mg/dl but the amniotic fluid cultures were positive. In this group there were four patients with amniotic fluid glucose levels <22 mg/dl. Two of these patients had a positive Gram stain and were delivered. The other two patients were delivered within 48 hours of amniocentesis without clinical evidence of maternal or neonatal infection. In the remaining 10 patients the amniotic fluid glucose concentration was ≥24 mg/dl and the amniotic fluid cultures were positive for *U. urealyticum* alone. All of these cases occurred in patients with premature rupture of membranes, in whom cervical cultures were also positive for U. urealyticum.

Clinical chorioamnionitis developed within 48 hours

Table I. Comparison of amniotic fluid glucose and Gram stain

	Amniotic fluid glucose level ≤16 mg/dl	Gram stain (%)
Sensitivity	79.1	29.9
Specificity	94.2	97.8
Positive predictive value	86.9	87.0
Negative predictive value	90.2	74.0

after the amniocentesis in 22 patients (10.7%). Amniotic fluid cultures were positive in 19 of these patients. The Gram stain was positive in two of the three patients with negative amniotic fluid cultures. Amniotic fluid glucose concentration was ≤16 mg/dl in 21 of the patients who subsequently developed clinical chorioamnionitis. The remaining patient had a glucose concentration of 17 mg/dl, a positive Gram stain, and a positive culture. There were no cases of documented neonatal sepsis in the study population.

Comment

The results of this study demonstrate that in patients ≤34 weeks' estimated gestational age with preterm labor or premature rupture of membranes, the amniotic fluid glucose concentration was significantly lower in those with positive amniotic fluid cultures than in those with negative cultures. This finding is consistent with the only other published report on amniotic fluid glucose concentration in intraamniotic infection by Romero et al.11 They performed amniocentesis on 168 consecutive patients with preterm labor and intact membranes. Amniotic fluid cultures were positive in 23 patients (13.6%). An amniotic fluid glucose value of <14 mg/dl had a sensitivity of 87%, a specificity of 92%, a positive predictive value of 63%, and a negative predictive value of 98% in the detection of a positive amniotic fluid culture. The higher positive predictive value in our study (87%) most likely is due to the higher prevalence of positive amniotic fluid cultures (32.8%).

In this study an amniotic fluid glucose concentration of ≤16 mg/dl predicted subclinical intraamniotic infection significantly better than Gram stain. The sensitivity of the Gram stain in our study is lower than what is generally reported in the literature. 1-5, 8, 9, 11 However, many of these studies did not culture for Mycoplasma species, which are not visible on Gram stain. Our results compare somewhat more favorably with a study by Romero et al.8 in which cultures for Mycoplasma species were performed. They reported a sensitivity of 44.8% for the Gram stain. The false-positive rate was higher with amniotic fluid glucose determinations than with Gram stain (5.8% vs 2.2%, respectively). If amniotic fluid glucose concentration is to be used clinically, the false-positive rate must be low to avoid the unnec-

Table II. Patients with amniotic fluid glucos	e levels ≤16 mg/dl and	l negative amniotic Hi	aid culture results
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Patient No.	Indication for amniocentesis	Estimated gestational age (wk)	Amniotic fluid glucose	Gram stain	Maternal glucose (mg/dl)	Comments
1	Preterm labor	26	2	Many white blood cells Gram-negative rods	. 75	No tocolysis—positive Gram stain; subsequent clinical chorioamnionitis
2	Prematue rup- ture of mem- branes	31	3	Moderate white blood cells Gram-positive cocci	88	Labor induced — positive Gram stain; subsequent clinical chorioam- nionitis
3	Preterm labor	24	14	Negative	93	Failed tocolysis
4	Preterm labor	33	14	Few white blood cells	89	Failed tocolysis
5	Preterm labor	32	14	Rare white blood cells	36	Successful tocolysis; low maternal glucose
6	Preterm labor	28	14	Negative	80	Failed tocolysis; prob- able abruptio pla- centae
7	Preterm labor	32	14	Rare white blood cells	109	Failed tocolysis; prob- able abruptio pla- centae
8	Preterm labor	32	15	Rare white blood cells	69	Failed tocolysis; sub- sequent clinical chorioamnionitis

essary delivery of a premature infant. In reviewing our eight cases of false-positive results, two cases (patients 1 and 2 in Table II) most likely represent instances of false-negative cultures. Both of these patients had a positive Gram stain, and clinical chorioamnionitis developed within 12 hours of the amniocentesis. Five of the six remaining patients had preterm labor that was unresponsive to tocolysis. These cases may represent instances of subclinical infection of the decidua, placenta, or membranes where the microorganisms had not yet reached the amniotic cavity. The remaining false-positive result occurred in a patient with a serum glucose concentration of 36 mg/dl.

Several factors have been associated with decreased amniotic fluid glucose levels, including intrauterine growth retardation, preeclampsia, low maternal glucose, and advanced gestational age.12-14 Weiss et al.12 demonstrated that amniotic fluid glucose concentration decreased as gestational age increased, with a mean of 26 mg/dl at 34 weeks, which decreased to 15 mg/dl at 40 to 42 weeks. The lower 10th percentiles were 15 and 9 mg/dl, respectively. These data, together with a less aggressive management of preterm labor and premature rupture of membranes in patients >34 weeks' estimated gestational age at our institution, led to the decision to exclude patients >34 weeks' estimated gestational age from our study. When intrauterine growth retardation, preeclampsia, or low maternal glucose is present, caution is necessary when the amniotic fluid glucose concentration is interpreted because false-positive results theoretically may occur more frequently in these situations.

The ideal management of preterm labor and premature rupture of membranes is controversial. The most widely accepted management in patients ≤34 weeks' estimated gestational age is to prolong the pregnancy, either with tocolytics in preterm labor or with conservative management in premature rupture of membranes. ^{15, 16} Thus false-negative results of amniotic fluid glucose concentration would be clinically less problematic because the usual management would not be affected. However, the mother and fetus may be at increased risk of infectious sequelae.

In this study there were 14 patients with false-negative results (Table III). In two patients the Gram stain was positive, and they were delivered. False-negative results occurred in 11 patients who had premature rupture of membranes and the culture was positive for *U. urealyticum* alone. Although the cause of a decreased amniotic fluid glucose level in subclinical intraamniotic infection is not known, this finding is not unexpected because *U. urealyticum* does not use glucose as a substrate. In these 11 patients there were no cases of clinical chorioamnionitis or neonatal sepsis. Therefore in patients with premature rupture of membranes an amniotic fluid culture that is positive for *U. urealyticum* alone may not be an indication to deliver a premature infant

Diabetes, tocolysis with a β -adrenergic receptor agonist, and betamethasone administration were associated with the highest amniotic fluid glucose concentrations in patients with negative amniotic fluid cultures. This included eight of the nine patients who had an amniotic fluid glucose level >60 mg/dl. This association

Table III. Patients with amniotic fluid glucose levels >16 mg/dl and positive amniotic fluid culture results

Patient No.	Indication for amniocentesis	Estimated gestational age (wk)	Aniotic fluid glucose (mg/dl)	Maternal glucose (mg/dl)	Gram stain	Culture	Comments
1	Premature rup- ture of mem- branes	26	17	86	Few white blood cells Gram-negative rods	Escherichia coli	Labor induced—positive Gram stain
2	Preterm labor	26	18	128	Moderate white blood cells Gram-negative cocci Gram-positive rods	Polymicrobial*	Tocolysis discontinued—positive Gram stain
3	Premature rup- ture of mem- branes	34	. 19	80	Few white blood cells	U. urealyticum	Delivered 2 days later; no evidence of in- fection
4	Premature rup- ture of mem- branes	33	21	108	Moderate white blood cells	K. pneumoniae	Labor induced—positive culture; repeat glucose measurement 16 hr later—4 mg/dl†
5	Premature rup- ture of mem- branes	30	24	140	Rare white blood cells	U. urealyticum	Delivered 16 days later; evidence of infection‡
6	Premature rup- ture of mem- branes	32	31	87	Rare white blood cells	U. urealyticum	Delivered 10 days later; no evidence of in- fection‡
7	Premature rup- ture of mem- branes	32	31	95	Negative	U. urealyticum	Delivered 5 days later; no evidence of in- fection‡
8	Premature rup- ture of mem- branes	33	32	83	Few white blood cells	U. urealyticum	Delivered 2 days later; no evidence of infection‡
9	Premature rup- ture of mem- branes	32	34	60	Negative	U. urealyticum	Delivered 6 days later; no evidence of in- fection‡
10	Premature rup- ture of mem- branes	31	37	93	Rare white blood cells	U. urealyticum	Delivered 10 days later; no evidence of infection‡
11 -	Premature rup- ture of mem- branes	33	43	74 .	Rare white blood cells	U. urealyticum	Delivered 4 days later; no evidence of infection‡
12	Premature rup- ture of mem- branes	28	45	75	Few white blood cells	U. urealyticum	Delivered 26 days later; no evidence of infection‡
13	Premature rup- ture of mem- branes	24	46	53	Negative	U. urealyticum	Amniocentesis 14 days later—preterm labor; positive Gram stain and culture (Bacteroides fragilis); no tocolysis
14	Premature rup- ture of mem- branes	26	62	166	Rare white blood cells	U. urealyticum	Amniocentesis 4 days later—preterm labor; positive Gram stain and culture (<i>Proteus mirabilis</i>); no tocolysis

^{*}Lactobacillus, Peptostreptococcus magnus, a-hemolytic Streptococcus, Neisseria gonorrhoeae.

did not appear to be present in patients with positive amniotic fluid cultures. With the exception of patient 14 in Table III, who received terbutaline, all the diabetic patients and the patients who received a \beta-adrenergic receptor agonist or betamethasone with a positive amniotic fluid culture also had an amniotic fluid glucose level ≤16 mg/dl. However, until further research is done, caution should be exercised when amniotic fluid glucose concentration is used to assess for subclinical chorioamnionitis in patients with hyperglycemia.

In our study an amniotic fluid glucose concentration

of <13 mg/dl had a positive predictive value of 100% if patients 1 and 2 in Table II are considered to have false-negative amniotic fluid cultures. Similarly, a negative predictive value of 100% could be obtained for an amniotic fluid glucose concentration ≥22 mg/dl if patients without symptoms who had an amniotic fluid culture that was positive for U. urealyticum alone are excluded. In spite of this manipulation of the data, a gray zone of amniotic fluid concentrations exists between 13 and 21 mg/dl, which limits the clinical usefulness of the test. The serial amniotic fluid glucose

[†]Same amniotic fluid sample kept refrigerated.

[‡]No clinical evidence of maternal infection and no clinical or laboratory evidence of neonatal sepsis.

results obtained from patient 4 in Table III may indicate a possible method of improving the test. In this case a repeat amniotic fluid glucose concentration was done on the same sample, which was kept refrigerated, 16 hours after the initial determination. The amniotic fluid glucose concentration decreased from 21 to 4 mg/dl and the culture was ultimately positive for Klebsiella pneumoniae.

Amniotic fluid glucose concentration appears to be a rapid and sensitive test for detecting subclinical intraamniotic infection in pregnancies ≤34 weeks complicated by preterm labor or premature rupture of membranes. Further research is needed to determine the cause of the low amniotic fluid glucose levels found in association with intraamniotic infection and to develop modifications that improve the accuracy of the test in predicting amniotic fluid culture results.

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Rate of recurrence of preterm premature rupture of membranes in consecutive pregnancies

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The reported incidence of preterm premature rupture of membranes ranges between 1% and 2% of all pregnancies. The rate of recurrence is poorly defined. The goal of this study was to establish the frequency of recurrence in a high-risk referral practice. Over a 5-year period we identified 121 patients with preterm premature rupture of membranes who had a minimum of two consecutive pregnancies under our care, resulting in a total of 255 pregnancies for analysis. Recurrent preterm premature rupture of membranes occurred in 39 of 121 patients, for a rate of 32.2% (95% confidence interval, 23.9 \pm 40.5). We were unable to demonstrate an association between the estimated gestational age at the time of rupture in the index pregnancy, latency period, interval between pregnancies, and the probability of repeat preterm premature rupture of membranes in the next pregnancy. We conclude that patients with preterm premature rupture of membranes should be counseled regarding the significant risk of recurrence and need to have close follow-up in their subsequent pregnancies. (AM J OBSTET GYNECOL 1991;165:1111-5.)

Key words: Preterm premature rupture of membranes, recurrence

Preterm premature rupture of membranes occurs in approximately 1% to 2% of all pregnancies.^{1, 2} The cause remains unknown in most cases, although many predisposing conditions have been proposed, including factors such as incompetent cervix, amnionitis, diethylstilbestrol exposure, uterine anomalies, multiple gestations, and trauma.

In many populations preterm premature rupture of membranes is the most commonly identified factor associated with preterm deliveries, occurring in >30% of all such births.^{3,4}

In counseling such patients we are currently unableto tell them the risk of recurrence of preterm premature rupture of membranes, yet in patients with less common causes of prematurity such as idiopathic preterm labor, abruptio placentae, fibroids, and polyhydramnios, we have accurate information from multiple studies.⁵⁻¹⁰ Therefore this study was pursued to close this important gap in our knowledge.

Material and methods

A retrospective analysis was conducted at Long Beach Memorial Women's Hospital of all patients de-

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Table I. Characteristics of index pregnancies

Estimated gestational age at pre- term premature rupture of membranes (index pregnancy) (wk)	31.4 ± 4.9
Latency period (index pregnancy)	5.5 ± 14.6
(days) Interval between pregnancies (mo)	28.5 ± 13.4

Data mean ± SD.

livered with the diagnosis of preterm premature rupture of membranes between 1983 and 1987. Preterm premature rupture of membranes was defined as rupture of membranes before 36 completed weeks. In all patients rupture of membranes was documented by sterile speculum examination with pooled fluid, ferning, and alkaline pH determination (Nitrazine paper). Best estimated gestational ages were derived from the last menstrual period and ultrasonographic examination on admission.

Patients with the diagnosis of incompetent cervix, uterine anomalies, diethylstilbestrol exposure, and multiple gestation and neonates with congenital anomalies were excluded from the data pool. During the study period 1050 patients with preterm premature rupture of membranes were identified; 121 met the inclusion criteria and had consecutive pregnancies under our care at Long Beach Memorial Medical Center, resulting in a total of 255 pregnancies for analysis.

Descriptive statistics are presented as the mean \pm 1 SD. Data were analyzed by means of χ^2 with Yates' correction, Fisher's exact test, and the Student t test. All comparisons were against a two-sided alternative

Table II. Demographic comparisons

	No preterm premature rupture of membranes in next pregnancy $(n = 82)$	Preterm premature rupture of membranes in next pregnancy (n = 39)
Maternal age (yr)	27.6 ± 5.2	26.0 ± 5.4
Gravidity	2.4 ± 5.2	$2:3 \pm 1.2$
Parity '	0.5 ± 0.9	0.7 ± 0.9
Estimated gestational age at preterm premature rupture of membranes (wk)	31.5 ± 4.7	30.9 ± 5.1
Estimated gestational age at delivery (wk)	32.4 ± 4.3	31.5 ± 4.7
Latency (days)	6.2 ± 16.1	3.9 ± 10.6
Interval (mo)	28.7 ± 13.3	28.1 ± 13.6
Next estimated gestational age at delivery (wk)	38.6 ± 3.0	33.0 ± 5.1

Data are mean ± SD.

hypothesis, and a p value of <0.05 was considered statistically significant.

Results

Of the 121 patients evaluated, 110 underwent followup for one subsequent pregnancy and 11 patients had follow-up of two or more subsequent pregnancies, resulting in a total of 255 pregnancies. Preterm premature rupture of membranes recurred in 39 patients, for a rate of 32.2% (95% confidence interval, 23.9 to 40.5). As shown in Table I the average estimated gestational age at preterm premature rupture of membranes in the index pregnancy was \$1.4 ± 5.1 weeks. The average latency period was 5.5 ± 14.6 days, and the mean interval between pregnancies was 28.5 ± 13.4 months. Table II compares demographic and other characteristics between the group of patients without preterm premature rupture of membranes in their next pregnancy and those with recurrence. The differences observed did not achieve statistical significance. We then analyzed the effect of the length of the latency period in the index pregnancy on the probability of recurrence of preterm premature rupture of membranes in the next pregnancy; we were unable to demonstrate an association (Fig. 1). A similar analysis looking at the interval between pregnancies (Fig. 2), the gestational age at delivery in the index pregnancy (Fig. 3), and the gestational age at preterm premature rupture of membranes in the index pregnancy (Fig. 4) failed to show any significant associations with the risk of recurrence.

Comment

The issue of recurrence of preterm premature rupture of membranes in consecutive pregnancies has remained essentially unexplored. A 15-year review of the literature yielded only one such study performed by Naeye¹¹ in 1982. He reviewed the course of consecutive pregnancies of 5230 women who had been enrolled in the Collaborative Perinatal Project of the National In-

stitute of Neurological and Communicative Disorders and Stroke between 1959 and 1966. The total number of pregnancies analyzed was 10,460; however, the incidence of preterm premature rupture of membranes was not given. The rate of recurrence in patients with preterm premature rupture of membranes in their index pregnancies was 21%, as compared with only 4% when the index pregnancy went to term. A history of preterm delivery without rupture of membranes in the initial pregnancy was associated with a 10% risk of preterm premature rupture of membranes in the next pregnancy. The stated purpose of Naeye's study was to evaluate the factors that predispose to premature rupture of membranes. It was not specifically designed to address the question of recurrence. In addition, it is limited by the inherent errors in accurately assessing the estimated gestational age and the incidence of preterm premature rupture of membranes in such a wide collaborative study involving 12 centers and spanning 7 years.

In our study estimated gestational ages were calculated from the last menstrual period and were confirmed by an ultrasonographic examination, on admission in every case. By excluding patients with incompetent cervix, history of diethylstilbestrol exposure, uterine anomalies, and multiple gestations and fetuses with congenital anomalies, we tried to define a subgroup of patients with true idiopathic preterm premature rupture of membranes. Whereas we did not correlate the cervicovaginal microbiologic flora with the risk of recurrence, it is important to note that none of the 39 patients with recurrence had evidence of clinical amnionitis at the time of rupture of membranes in the second pregnancy. Surprisingly, we were unable to demonstrate an association between the estimated gestational age at the time of rupture in the index pregnancy and the probability of recurrence in the next pregnancy. Similarly, we were not able to show that patients with a longer latency period in the index preg-

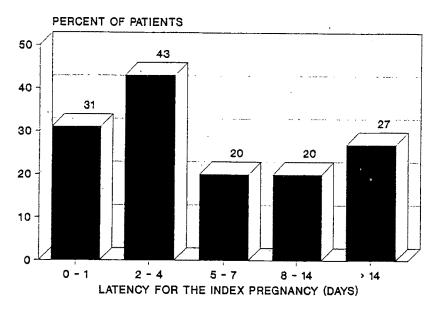


Fig. 1. Percent of patients with recurrent preterm premature rupture of membranes by latency period in index pregnancy.

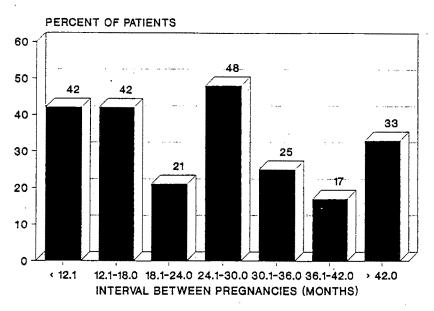


Fig. 2. Percent of patients with recurrent preterm premature rupture of membranes by interval between pregnancies.

nancy were less likely to have recurrent preterm premature rupture of membranes. The estimated gestational age at the time of preterm premature rupture of membranes in the index pregnancy of patients with recurrence was 30.9 ± 5.1 weeks, as compared with 33.0 ± 5.1 weeks at the time of the recurrence. Although the difference did not reach statistical significance (p=0.07), there was a strong trend suggesting that patients with recurrent preterm premature rup-

ture of membranes will have ruptured membranes at a later gestational age in the next pregnancy. The main limitation of this analysis is its low "capture" rate, having only 121 patients return to our care for their subsequent pregnancies. However, these patients returned to our hospital of their own accord and were not referred back by their private physicians. This, we believe, eliminates any selection bias that may have occurred. There is no apparent reason why patients who were

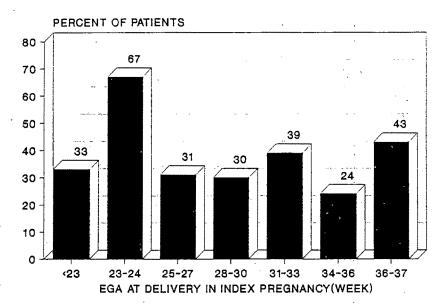


Fig. 3. Percent of patients with recurrent preterm premature rupture of membranes by estimated gestational age at delivery in index pregnancy.

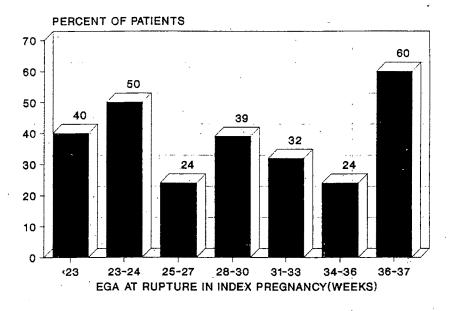


Fig. 4. Percent of patients with recurrent preterm premature rupture of membranes by estimated gestational age at rupture in index pregnancy.

delivered at our hospitals in their subsequent pregnancies would be different from patients delivered at other institutions.

Overall, the above data demonstrate that there is a significant tendency for preterm premature rupture of membranes to be repeated in consecutive pregnancies. We hope that this study will facilitate the counseling of patients with preterm premature rupture of membranes and help the clinician in identifying a group of patients in need of closer observation and

follow-up as part of an overall prematurity prevention program.

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Renal pelvicalyceal dilation in antepartum pyelonephritis: Ultrasonographic findings

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The purpose of the present study was to determine whether pregnant women with pyelonephritis have differences of pelvicalyceal systems, compared with normal pregnant control subjects, that might predispose to upper urinary tract infection. Ultrasonographic examination of both kidneys in coronal and axial planes of 24 women with clinical pyelonephritis and positive urine cultures was compared with results in control subjects matched for gestational age, parity, and race. Women with right or bilateral pyelonephritis had increased dilation of the right calyceal system, compared with controls (1.7 cm vs 0.8 cm, p < 0.001). Renal pelvis volume was increased as well (29.3 vs 5.5 cm³, p < 0.001). Renal pelvicalyceal dilation in antepartum pyelonephritis was significantly increased compared with normal physiologic dilation of pregnancy. Follow-up nephrosonography in a small number of women (N = 10) after treatment of pyelonephritis did not reveal a consistent decrease in renal dilation, suggesting that dilation of the renal pelvis may antedate pyelonephritis. Further study of this phenomenon is warranted. (AM J OBSTET GYNECOL 1991;165:1115-9.)

Key words: Pyelonephritis, nephrosonography, hydronephrosis

Physiologic dilation (hydronephrosis) of the collecting system of the kidneys during pregnancy is a well-described entity. 1-3 Various causes have been proposed for this urinary obstruction or stasis, including dextrorotation of an enlarging uterus and dilatory effects of progesterone on the ureters. 4-6 Nephrologic ultrasonography of hydronephrosis, or pelvicalyceal dilation, during pregnancy has been reported by several inves-

tigators.⁷⁻¹¹ Greater dilation of the right kidney collecting system, compared with that of the left collecting system, is well documented.⁸⁻¹¹

Pyelonephritis complicates approximately 1% to 2% of pregnancies, although its incidence has decreased with routine detection of asymptomatic bacteriuria. ¹² Symptomatic pyelonephritis occurs more frequently in the right kidney, and this unilateral pattern appears to be related to urinary stasis and reflux. ^{12, 13} Certain characteristics of some of the infecting organisms play a role in the development of pyelonephritis. It is now recognized that the fimbriae of *Escherichia coli* promote adhesion to the urinary tract epithelium, allowing the bacteria to invade and multiply. This frimbria-specific, O-antigen type *E. coli* is the major pathogen in ascending pyelonephritis. ^{13, 14} Urinary stasis and pyelonephritis are clearly associated, but the correlation of upper

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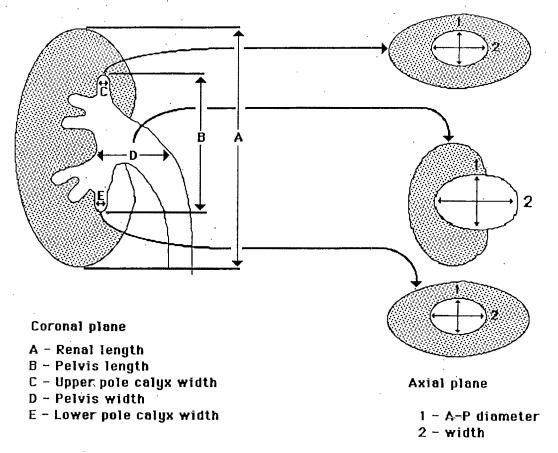


Fig. 1. Three-dimensional renal anatomy and parameters measured during nephrosonography.

urinary tract infections with the degree of collecting system dilation has not been delineated in pregnancy. Reports in the literature are conflicting, primarily because the renal collecting system dilation examined did not select exclusively for pyelonephritis but included urinary tract problems of various causes, including asymptomatic bacteriuria, and renal calculi.^{8, 11} The purpose of our prospective study was to determine whether pregnant patients with clinical pyelonephritis had renal pelvicalyceal dilation that was greater than normal physiologic dilation of pregnancy. Additional ultrasonographic characteristics of the kidney also were assessed.

Material and methods

Patients. Fifty-three pregnant women underwent 65 ultrasonographic examinations between July 1989 and September 1990. Twenty-seven women were admitted to the hospital with the diagnosis of acute pyelone-phritis. The criteria for this diagnosis were elevated temperature, flank pain, pyuria, and positive urine culture. White blood cell count, creatinine, and urine red blood cell count were recorded. Two of these women were readmitted for treatment of recurrent pyelone-phritis. Renal sonograms were obtained in these pa-

tients, for a total of 29 ultrasonographic studies within 24 hours of admission. Of these 29 examinations of 27 patients, two studies were eliminated when urine cultures were subsequently reported as negative and nephrosonography revealed renal stones. One ultrasonographic study was eliminated because crossedfused ectopia of the collecting system was diagnosed, for a total of 26 examinations of 24 patients included in our study. These 24 women were matched to controls for gestational age within 2 weeks (on the basis of ultrasonographic biometry), race, and parity. The controls were chosen on the basis of negative history for renal disease or treatment for asymptomatic bacteriuria, for which we routinely screen in our population. The confirmation of gestational age by ultrasonographic biometrics instead of last menstrual period was thought to be more reliable in our patient population. In addition, nine patients underwent 10 repeat sonography after intravenous hydration and antibiotic therapy were discontinued.

Ultrasonographic methods. Renal ultrasonographic examinations were performed with women placed in the right and left lateral decubitus positions with an UltraMark IV or VIII (ATL, Bothell, Wash.) ultrasonographic scanner equipped with 3 and 5 MHz me-

Table I. Measurement of renal collecting system

	Patients with pyelonephritis		Control subjects $(N=25)$		
	Mean	SE	Mean	SE	p Value*
Patients with right or bilateral				-	
flank tenderness ($n = 24$)					
Renal pelvis volume†	29.3	5.3	5.5	1.2	< 0.001
Calyceal dilation—coronal‡	1.7	0.1	0.8	0.1	< 0.001
Áxial area§	3.8	0.6	0.9	0.2	< 0.001
Patients with right tenderness					
only $(n = 20)$					
Renal pelvis volume†	5.9	0.8	2.2	0.3	< 0.001
Calyceal dilation—coronal‡	0.8	0.1	0.6	0.0	0.03
Axial area§	0.5	0.2	0.3	0.0	0.27

^{*}Analysis of variance.

chanical sectors or annular transducers. Pelvicalyceal dilatation and renal pelvis volumes were determined in coronal and axial planes of the kidneys after bladders were empty (Fig. 1). Patients with full bladders voided. In the coronal plane the renal pelvis length, kidney length, and greatest widths of the upper-pole calyx, lower-pole calyx, and renal pelvis were determined. In the axial plane the greatest anteroposterior and width dimensions of the upper and lower pole calyces and renal pelvis width were measured. The greatest pelvicalyceal dilation in the coronal and axial planes were compared. In addition, renal pelvis volume was determined by the ellipsoid formula proposed by Cietak9: Volume = Length × Width × Thickness (anteroposterior plane) × 0.5223. Measurements of the renal pelvis in our study were altered from those of Cietak and Newton not to include the entire echogenic portion of the pelvis; only those sonolucent regions within the pelvis that were thought to represent the collecting system were included (Fig. 1). The overall appearance of the renal parenchyma was assessed to evaluate for any focal lesions or changes in general pattern of echogenicity.

Of the 26 studies performed on symptomatic patients with urine culture-positive pyelonephritis, 20 had right-sided symptoms, four had bilateral symptoms and two had left-sided symptoms. Ultrasonographic measurements of the collecting system in patients with right and bilateral symptoms (n = 24) were compared with those of matched controls by means of analysis of variance with the Student-Newman-Keuls test. In addition, repeat sonograms (n = 10), which were obtained in nine women with pyelonephritis after the discontinuation of intravenous hydration and antibiotics, were evaluated with paired test analysis. A statistical analysis was performed with SAS version 6.04 (Statistical Analysis System, SAS Institute, Cary, N.C.) on an AST 386 (AST Research, Inc., Irvine, Calif.) computer.

Results

Of the 24 patients with pyelonephritis, nine were white, nine were Hispanic, and six were black. Fourteen patients were nulliparous, whereas 10 patients had had one or more children. Sixteen patients had body temperatures >38° C, whereas eight had normal temperatures during hospitalization. Eighteen patients had an elevated white blood cell count, i.e., $>10.9 \times 10^9/L$. Eighteen catheterized urine cultures were positive for E. coli (>10⁵ organisms), three had Proteus mirabilis (>10⁵ organisms), and three had Klebsiella pneumonice (>10⁵ organisms). Serum creatinine was 1.0 mg/dl in one patient and 1.2 and 1.3 mg/dl in another patient with pyelonephritis on two separate admissions, while the creatinine level of the remaining 22 patients fell below 1.0 mg/dl.

Of the 26 nephrosonographic examinations performed on 24 patients, results in 24 were compared with those of matched controls in those patients with right (n = 20) or bilateral (n = 4) flank pain. Among pregnant women with pyelonephritis involving the right or both kidneys (n = 24), the right renal pelvis volume was significantly increased compared with that of matched pregnancy controls (29.3 vs 5.5 cm³, p < 0.001). In addition, the maximal calyceal dilation was significantly increased (1.7 vs 0.8 cm, p < 0.001) (Fig. 2 and Table I). Maximal area dilation of the calyceal system in the axial plane also was significantly increased among patients with symptoms compared with controls (p < 0.001).

Patients with acute pyelonephritis and left flank pain (n = 2) or bilateral findings (n = 4) were not analyzed because of small numbers. A comparison of left-kidney

[†]Renal pelvis volume (in cubic centimeters) = Length × Width × Anteroposterior diameter × 0.523.

[‡]Maximal dilation in coronal plane (in centimeters).

Axial plane area (in square centimeters) = Anteroposterior diameter \times Width.

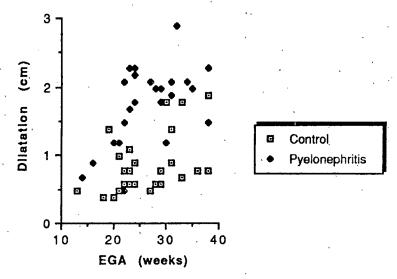


Fig. 2. Maximum calyceal dilation of right kidney in coronal plane versus gestational age. GA, Estimated gestational age.

pelvicalyceal measurements in patients with only right-sided acute pyelonephritis (n=20) compared with those of matched controls revealed that the left renal pelvis volume was significantly increased in patients with symptomatic right pyelonephritis (5.9 vs 2.2 cm³, p < 0.001). A statistically significant difference in maximal calyceal dilation between the patient and control groups occurred, but because the actual difference was <3 mm, the result was not clinically significant. No statistically significant difference was found in the area of maximum dilation in the axial plane of the calyces on the left side between patients with only right-sided symptoms and controls.

Nine women underwent 10 follow-up nephrosonographic examinations between 2 and 30 days after termination of intravenous antibiotics and hydration. In four cases the maximal calyceal dilation on the symptomatic side increased between 1 and 3 mm, when compared with the hospital admission sonogram. In three cases the calyceal dilation decreased between 1 and 4 mm, and there was no change in three cases. The renal pelvis volume of the right and left sides demonstrated no statistically significant change with *t* test analysis. Assessment of the ultrasonographic characteristics of the renal parenchyma revealed no focal or global changes. Focal nephritis and perinephric fluid collections were not apparent.

Comment

Stasis in the urinary collecting system and the development of an ascending urinary tract infection are clearly associated, but consistent findings of renal pelvis dilation in association with acute pyelonephritis in this study are new. In our study the clinical presentation of pyelonephritis is similar to that previously reported with right-sided symptoms occurring most frequently.

followed less commonly by bilateral symptoms and then left-sided symptoms, which are the least common. Increased dilation of the right collecting system in pregnant patients with right or bilateral pyelonephritis compared with that of matched controls was statistically significant. This was demonstrated in the maximal polar pelvicalyceal dilation in the coronal plane, the maximal polar pelvicalyceal area in the axial plane, and pelvicalyceal volumes, determined by the ellipsoid formula.

Interestingly, there was a statistically significant increased dilation of the left renal pelvis in patients with right-sided pyelonephritis, although the actual leftsided volumes were much less than the right-sided dilation. However, this was not reflected in the polar pelvicalyceal measurements. As a possible explanation of these findings, patients with pyelonephritis have bilateral renal pelvis dilation with the right more dilated than the left. Greater dilation on the right side would possibly increase the likelihood that this side would become infected with an ascending process. Alternatively, both kidneys were infected, but infection on the left side was not as severe as that on the right; therefore symptoms were less severe on the left. This latter speculation is actually supported by radiologic findings in one study, which found bilateral renal abnormalities with intravenous pyelograms in spite of clincial symptoms on one side.15 It was concluded that there may be clinically silent involvement of the opposite kidney.

Three patients were eliminated from the study group because of renal complications other than pyelonephritis. Ultrasonography detected previously unknown renal pelvis calculi in two of these patients and one congenital anomaly, fused pelvic kidneys. The findings had important implications for clinical management.

Patients being treated for pyelonephritis are given intravenous antibiotics and hydration.16 The effect of intravenous hydration on the collecting system may confound the findings of our study. Adequate hydration should not affect the upper urinary tract dimensions if the bladder is empty.17 The normal creatinine level in all but two patients suggests adequate hydration. Our follow-up evaluations of nine pregnant patients with 10 sonograms, after the intravenous supplementation and antibiotic therapy were discontinued, demonstrated no trend in a small series. Renal pelvis volume of either side did not change, and the pelvicalyceal dilation of the upper or lower pole of the involved kidney(s) changed very little, a difference probably not greater than measurement error (≤ 3 mm).

There is extensive mention in the literature about the radiographic appearance of pyelonephritis in nongravid patients. Early studies based on excretory nephrograms revealed normal kidneys in 75% to 80% of patients.15 Most abnormalities were parenchymal changes with pelvic dilation in five of 40 patients and calyceal dilation in three of 40 cases. In six patients undergoing computed tomography and ultrasonography in one study¹⁸ and two patients having ultrasonography in another,19 no hydronephrosis was seen. Friedland et al.20 believe hydronephrosis is not present with ultrasonography in acute pyelonephritis and, if seen, should raise the possibility of pyonephrosis, a serious complication with implications for management, including drainage. These findings suggest hydronephrosis on ultrasonography is an uncommon sequela of acute pyelonephritis in the nonpregnant patient population.

It appears that hydronephrosis, more than the normal physiologic dilation of pregnancy, is found in antenatal pyelonephritis; this hydronephrosis is rarely seen as an ultrasonographic finding in the nonpregnant population. Given the knowledge that physiologic dilation of pregnancy is commonplace and pyelonephritis is increased in the pregnant populations, one may speculate that a greater degree of dilation may be a risk factor for development of pyelonephritis.

The results of this study have important clinical implications. If the dilation antedates the onset of pyelonephritis, the potential for screening populations at risk may exist. A further longitudinal study of patients with serial nephrosonography of a randomly selected cohort of pregnant women to determine whether the dilation

precedes the development of upper urinary tract infection is needed.

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Doppler flow velocities in single umbilical arteries

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Fetal compromise has been associated with an umbilical artery waveform pattern of low or absent diastolic velocity relative to systolic velocity. Fetuses with single umbilical arteries have an increased risk of major malformations, mortality, retarded fetal growth, and prematurity. In this study Doppler flow velocities were obtained in 13 fetuses (four twin fetuses and nine singletons) with a single umbilical artery. Five (38%) fetuses, consisting of four singletons and one twin, had anomalies. Six (46%) fetuses were small for gestational age, including two twin fetuses and three singleton fetuses with anomalies. Three (23%) of the 13 systolic-to-diastolic velocity ratios were abnormally high. Whereas this is a higher rate of abnormal ratios than the reported 2% to 3% in control populations, it is interesting to note that 77% of fetuses with single umbilical arteries had normal systolic-to-diastolic ratios. (AM J OBSTET GYNECOL 1991;165:1120-2.)

Key words: Single umbilical artery, Doppler flow velocities

Doppler ultrasonography has provided a means to perform noninvasive assessments of blood flow in fetal and placental vessels.1-6 As pregnancy progresses, there is a steady decline in umbilical artery ratios of systolicto-diastolic velocities. This decline has been attributed to a decrease in the resistance of umbilical circulation.4 It has been demonstrated that fetal growth retardation may be associated with persistently elevated systolic-todiastolic ratios after 30 weeks.1, 3, 5 As more umbilical artery flow velocity data become available from an everexpanding list of pathologic states, such as trisomy 13 and 18 and dizygotic and monozygotic twins, 4, 6 the question arises whether there is a high relative risk for abnormal systolic-to-diastolic ratios in single umbilical arteries compared with three-vessel cords.

Fetuses with a single umbilical artery are at increased risk for unfavorable outcome.7-10 In a study by Bryan and Kohler,10 143 of 20,000 infants had a single umbilical artery, giving a rate of 0.72%. Of these, 18% had major malformations, 34% were small for gestational age (SGA), and 17% were delivered preterm. Given the association of a single umbilical artery and the increased potential for perinatal morbidity and mortality, we examined Doppler flow velocities in two-vessel umbilical cords.

Material and methods

Between April 29, 1987, and Dec. 11, 1990, during which 10,349 ultrasonographic studies were per-

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Presented at the Eleventh Annual Meeting of the Society of Perinatal Obstetricians, San Francisco, California, January 28-February 2,

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formed, 13 patients with single umbilical arteries were examined with two-dimensional and Doppler ultrasonography. All studies were performed with a 3.5 or 5.0 MHz transducer. The indications for ultrasonography on those patients with a single umbilical artery included twin gestation for concordant growth, low maternal αfetoprotein levels, fundal height less than dates, and family history of encephalocele. Gestational age at the time of ultrasonography varied from 22 to 38 weeks. Single umbilical artery on ultrasonography was an incidental finding, and in most instances serial Doppler flows were not obtained. In three fetuses serial values were obtained, and in these cases the most recent result was reported. The systolic-to-diastolic ratios did not change significantly with time in this group. In twin gestations only the fetuses with a single umbilical artery showed an abnormal systolic-to-diastolic ratio; outcomes of twins with normal ratios are not described. In all instances standard ultrasonographic measurements were performed, including biparietal diameter, femur length, abdominal circumference, and amniotic fluid volume estimation. Gestational age was estimated from fetal size when menstrual dates were unknown or inaccurate. SGA was defined as birth weight <10th percentile by institutional standards. An abnormal Doppler systolic-to-diastolic ratio was defined as >95% confidence interval for gestational age.4 After identification of a single umbilical artery, the umbilical artery flow velocity waveform was obtained with a 3.5 MHz transducer with pulsed Doppler capability. With page-print outputs from a strip chart recorder, maximal velocity was measured from the 0 line to the highest part of the velocity peak (systole). Minimal velocity was measured at the nadir after the peak (diastole). A ratio of systolic peak to diastolic nadir was calculated. Umbilical artery pulsed Doppler waveforms were recorded for at least 8 to 10 beats. Fetuses were exposed to <46 mW/cm²

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Lable L. Patient	data in	pregnancies	with single	- umbilical arteri	z detected h	y ultrasonography
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EGA at Doppler flow study (wk)	No. of fetuses	SGA	Oligohydramnios	Anomalies	S/D ratio	Apgar 1 min	score 5 min	EGA at	Newborn intensive care unit admission
(wh)	140. Of Jesuses	30/1	Ougonyaramnios	Anomanes	SID Tatto	1 mun) min	aenvery	uamission
22	T	X	********	•	Reverse (high)	5	7	34	X
25	S	X	X	Triploidy	2.8	0	0	27	
25	T	X		Gastroschisis	4.1	8	9	35	X
28	S			*******	2.2	9	9	39	_
29	S				4.4 (high)	8	9	40	_
32	S			-	1.8	8	9	38	_
32	S		****	Encephalocele	3.0	7	9	35	X
32	T			-	4.7 (high)	6	8	36	_
34	T		anama.	*******	2.2	7	9	36	-
35	S	X	X .	Congenital cyto- megalovirus infection	2.0 .	7	8	38	X
35	S	X		Right renal agenesis	1.7	7	8	36	X
37	S	X	X		2.4	8	9	38	_
38	. S				2.7	9	9	40	. —

S, Singleton; T, twin; EGA, estimated gestational age; SD ratio, systolic/diastolic umbilical artery Doppler flow velocities.

power density according to manufacturer's specifica-

Management of the fetuses was carried out without knowledge about the Doppler information. At the time of birth, method of delivery, Apgar scores, weight, presence of meconium, presence of congenital anomalies, and admission to the neonatal intensive care unit were recorded.

Results

Nine singletons and four twins were included in this series. Maternal age was 23.9 ± 4.4 (mean ± SD), gravidity was 2.4 ± 1.3 , and parity was 1.4 ± 1.1 . Three of 13 (23%) patients underwent cesarean section. Indications for cesarean section were elective repeat cesarean section, failed trial of labor after previous cesarean section, and breech presentation. One fetus with a chromosome abnormality did not survive. There were no instances of fetal distress as defined by late decelerations detected by electronic fetal monitoring. As shown in Table I, in live-born infants, there were no 5-minute Apgar scores <7. Gestational age at the time of delivery was 36.4 ± 3.4 weeks with a mean birth weight of 2383 \pm 908 gm. In the twin fetuses there was one example of discordant growth in a fetus with a single umbilical artery and an elevated systolic-todiastolic ratio of 4.7. In spite of discordant growth the smaller twin was not SGA. Meconium was present in one instance in a breech delivery by cesarean section; this fetus had an systolic-to-diastolic ratio of 2.2.

Five of 13 (38%) of fetuses had congenital anomalies. None of these had abnormal systolic-to-diastolic ratios. The anomalies included gastroschisis, occipital encephalocele, congenital cytomegalovirus infection, right renal agenesis, and triploidy. Of six SGA fetuses, one had an elevated systolic-to-diastolic ratio. Of three fetuses with abnormally high systolic-to-diastolic ratios, one was SGA. The only intrauterine fetal death was in the fetus with a 69,XXY karotype at 25 weeks.

Comment

In this study we measured umbilical artery velocity in fetuses with a single umbilical artery. As pregnancy progresses, there is normally a steady decline in the umbilical artery systolic-to-diastolic ratio. This decline is theoretically caused by decreasing vascular resistance.4 A persistently elevated systolic-to-diastolic ratio may be associated with fetal growth retardation1. 2, 4, 5 and in some fetuses with congenital anomalies.8,6

It is well established that fetuses with a single umbilical artery are at increased risk for intrauterine growth retardation or congenital anomalies. Bernirschke⁸ found single umbilical arteries in 0.85% of singletons and 5% of twin gestations. Thirty-nine percent of the infants with one artery had associated anomalies. Other investigators quote a mortality rate as high as 14% in infants with a single umbilical artery.9 In addition, single umbilical arteries occur more often in diabetic pregnancies and in spontaneous abor-

In this small sample size there was no clear correlation between abnormally elevated systolic-to-diastolic ratios and SGA fetuses. The 17% sensitivity in this group is not surprising given the quoted sensitivity of Doppler in identifying growth retardation in large series of only 50% to 60%.4 It is interesting to note that none of the fetuses with congenital anomalies in this study had abnormal systolic-to-diastolic ratios, although

in larger series of fetuses with congenital anomalies the incidence of abnormal systolic-to-diastolic ratios is 50%. Three of the thirteen fetuses studied had abnormal systolic-to-diastolic ratios for a rate of 23%; this incidence is nearly 10 times the rate cited in control populations. It is interesting to note that in a situation in which the number of umbilical arteries was reduced from two to one, Doppler flow velocity ratios through these single umbilical arteries were normal in 77% of cases.

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Impact of prenatal testing on maternal-fetal bonding: Chorionic villus sampling versus amniocentesis

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The process of maternal-fetal attachment, considered vital for normal infant development, begins during pregnancy and can be affected by a number of external factors. In this study the impact of prenatal testing on maternal-fetal bonding was evaluated in 253 women undergoing either first-trimester chorionic villus sampling (n=101) or second-trimester genetic amniocentesis (n=152). The women were evaluated by means of a modification of the Cranley Maternal-Fetal Attachment Scale, administered before and after the results of the prenatal diagnostic testing were made known to them (mean gestational ages of 10.6 and 15.7 weeks for the chorionic villus sampling group and 16.5 and 21.1 weeks for the amniocentesis group). The results showed: (1) that maternal-fetal attachment begins as early as 10 weeks' gestation and increases significantly as the pregnancy progresses, (2) that maternal-fetal attachment increases significantly, once the results are known to be normal, for both groups (p < 0.001), (3) that this increase occurs about 5 weeks earlier for patients with chorionic villus sampling in comparison to those undergoing amniocentesis (p < 0.001). Thus, with regard to the process of maternal-fetal attachment, first-trimester chorionic villus sampling appears to be preferable to second-trimester amniocentesis. (AM J OBSTET GYNECOL 1991;165:1122-5.)

Key words: Maternal-fetal bonding, prenatal diagnosis, chorionic villus sampling, amniocentesis

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Reprint requests: J.M. Johnson, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, 200 Elizabeth St., 6EN-216, Toronto, Ontario, Canada M5G 2C4. 6/6/31198 The process of maternal-infant bonding, considered vital for normal infant development, has been shown to begin during pregnancy and can be affected by a number of external factors. ¹⁻³ While the bonding process in the later stages in pregnancy (>20 weeks' gestation) has been well investigated, ³⁻⁴ the extent to which bonding is manifest in early pregnancy (<20 weeks'

gestation) and the factors affecting this bond remain unclear. Prenatal diagnosis has been shown to have a significant effect on the emotional and psychologic components of pregnancy,5-7 but its impact on the bonding process in early pregnancy has not been previously investigated. Since the period of undergoing prenatal diagnosis and awaiting the results is associated with significant parental anxiety,8-10 it is possible that prenatal diagnosis may interfere with or delay this bonding process.

The purpose of this study was to evaluate maternalfetal bonding in early pregnancy to determine: (1) whether maternal-fetal bonding can be demonstrated before 20 weeks' gestation and (2) the effect of prenatal diagnosis on maternal-fetal bonding, specifically, whether bonding is delayed in patients who undergo amniocentesis as opposed to chorionic villus sampling (CVS).

Material and methods

The study group consisted of women with singleton pregnancies, confirmed by ultrasonography, with appropriate indications for prenatal testing according to the Canadian Guidelines for Prenatal Genetic Diagnosis.11 Eligible patients underwent nondirective genetic counseling regarding the risks, benefits, and limitations of chorionic villus sampling and/or amniocentesis and provided informed consent for their procedure of choice and for participating in the study.

The women were evaluated by means of a modification of the Cranley Maternal-Fetal Attachment Scale, ¹² administered before (preresult questionnaire) and after (postresult questionnaire) their results were made known to them. The Maternal-Fetal Attachment Scale allows patients to rank statements of their feelings about the pregnancy and the fetus in five areas of attachment: interaction with the fetus, giving of self, roletaking, differentiation from self, and attributing characteristics to the fetus. The statements are ranked on a scale from 1 to 5, with 5 being the most attached or bonded. In this study the scale was modified by omitting the category of "attributing characteristics to the fetus" because the questions were not appropriate for a gestational age <20 weeks.

The effect of ultrasonography on maternal attitudes toward the pregnancy and fetus were examined by asking the patient to rank a series of statements on a scale of 1 to 5 with 5 indicating the greatest effect.

The data were analyzed and statistical analysis, where appropriate, was by paired two-tailed Student t test with p < 0.05 being considered significant.

Results

Over the 4-month period beginning May 1989, 253 consecutive women undergoing chorionic villus sam-

Table I. Indications for prenatal testing

1		
Indication	Amniocentesis $(n = 151)$ $(\%)$	CVS (n = 102) (%)
Late maternal age	77	71
Previous abnormality	4 ·	19
Family history of genetic disease	4	10
Abnormal maternal serum α-fetoprotein level	15	N/A

NA, Not available.

pling (n = 101) or second-trimester genetic amniocentesis (n = 152) met the entry criteria. The groups did not differ significantly with respect to age, parity, socioeconomic status, or level of postsecondary education. Their indications for prenatal testing are described in Table I. A 100% follow-up rate was achieved in both groups.

The preresult and postresult questionnaires were administered to the patients at an average gestational age of 10.6 and 15.7 weeks for the CVS patients and at 16.5 and 21.1 weeks for the amniocentesis patients. This resulted in three comparison groups being generated: the preresult and postresult CVS group (group 1), the postresult CVS and preresult amniocentesis group (group 2), and the preresult and postresult amniocentesis group (group 3). In all three groups the level of bonding was significantly higher in the postresult group than in the preresult group (p < 0.001) (Table II). This increase in bonding occurred approximately 5 weeks earlier in patients undergoing CVS compared with those undergoing amniocentesis (Fig. 1).

There was an appreciable level of bonding (3.8 on a scale of 5) present in all preresult patients with no statistical difference between amniocentesis and CVS patients, in spite of a difference in gestational age of almost 6 weeks (Fig. 1). Similarly, the postresult levels of bonding did not differ significantly, with the postresult CVS patients achieving the same level of bonding by 15 weeks as the postresult amniocentesis patients achieved at 21 weeks (p < 0.001) (Table II).

All of the patients underwent one or more ultrasonographic examination(s) in association with their procedures. In 126 of 253 patients (50%), seeing the fetus on ultrasonography made them feel less anxious about the pregnancy and closer to the baby (72%), made the baby seem more real (87%) and in some cases made them feel "more like parents" (27%). As may be expected, positive feelings also made a significant percentage of the parents (55%) feel more anxious about the possibility of an abnormal result. These reactions were stronger at earlier gestational ages (≤12 weeks'

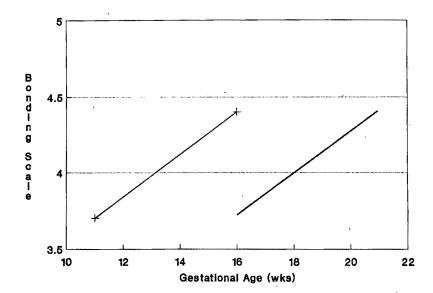


Fig. 1. Maternal-fetal bonding: CVS versus amniocentesis. Maternal-fetal bonding increased significantly in both CVS patients (thin line) and amniocentesis patients (thick line) once results of testing were reported to be normal (15.7 weeks' gestation for CVS patients, 21.1 weeks for amniocentesis patients). This increase in bonding occurred approximately 5 weeks earlier in CVS patients. Bonding scale is from 1 to 5 with 5 representing greatest bonding.

Table II. Maternal-fetal bonding: CVS versus amniocentesis

	Gro	up I	Gro	пир 2	Gro	up 3
Attachment task*	$Pre-CVS† \\ (n = 102)$	$Post-CVS^{\dagger}$ $(n = 102)$	$Post-CVS \\ (n = 102)$	$Pre-A\ddagger (n = 151)$	Pre-A $(n = 151)$	$Post-A\ddagger \\ (n = 151)$
Interaction with fetus Giving of self Role-taking Differentiation from self	2.7 4.2 3.8 4.6	3.5 4.8 4.5 4.8	3.5 4.8 4.5 4.8	2.8 4.3 4.0 4.4	2.8 4.3 4.0 4.4	3.6 4.8 4.7 4.9
Mean attachment	3.8	4.48	3.8	4.4§	3.8	4.5§

^{*}Modified Cranley Maternal-Fetal Attachment Scale (scale of 1 to 5, with 5 being most bonded).

gestation), although they also were present at later stages (13 to 20 weeks' gestation). We found that the first sonogram tended to have the greatest effect on their feelings toward the pregnancy, although subsequent sonograms also had an effect.

Comment

Our results indicate that in patients undergoing prenatal diagnosis, maternal-fetal bonding can be demonstrated as early as 10 weeks' gestation and increased significantly once the test results are known to be normal. This is consistent with previous studies that have shown that women undergoing prenatal diagnosis are reluctant to become "involved" in their pregnancies until their fetuses are known to be normal. This reluctance also may explain why, in these patients, bonding

appears more a function of the prenatal diagnosis experience than of gestational age. In this study the postresult CVS patients were shown to be significantly more bonded than were the preresult amniocentesis patients, in spite of being at a similar gestational age. Once the results were known to be normal, the level of bonding in CVS patients and amniocentesis patients was similar, suggesting that this factor plays a major role in the development of bonding in these patients. Previous investigators hve shown that CVS and amniocentesis patients have similar levels of attachment by the late second trimester,9 suggesting that CVS offers no long-term benefit in maternal-fetal bonding compared with amniocentesis. However, amniocentesis patients have been shown to be more bonded than gestational agematched controls once they know their fetuses are

[†]Preresult and postresult CVS patients.

[‡]Preresult and postresult amniocentesis patients.

p < 0.001, when compared with preresult bonding (paired two-tailed Student t test).

normal⁷; this seems to indicate that prenatal diagnosis may increase maternal-fetal bonding earlier in gestation. Maternal-fetal attachment has been shown to alter maternal life-styles to be more beneficial to the fetus³; therefore it is possible that an earlier increase in bonding may be beneficial by positively influencing maternal behavior at an earlier stage in pregnancy, when it may have a more significant effect on fetal outcome.

One of the factors that likely affects maternal-fetal bonding is maternal anxiety. Anxiety has been shown to correlate with the somatic symptoms of pregnancy and gestational age, as well as inversely with maternal education.18 Studies evaluating the effect of prenatal diagnosis on maternal anxiety have shown that CVS patients demonstrate an earlier reduction in maternal anxiety than amniocentesis patients.9, 10 The reduction parallels the increase in attachment we found and may have been a factor facilitating the ease and degree of bonding observed in our patients. The effect of prenatal diagnosis on third-trimester anxiety is unclear, although Marteau et al.6 reported that in patients who have undergone prenatal diagnosis, anxiety levels in the third trimester fall or remain the same as in the second trimester, whereas in the general population anxiety levels increase in the third trimester.

Preresult anxiety levels have been shown to be lower in patients whose primary indication for prenatal diagnosis is late maternal age compared with other indications.14 In this study later maternal age was the primary indication for prenatal diagnosis in 117 of 151 amniocentesis patients (77%) and 72 of 102 CVS patients (71%). Preresult binding levels in this subpopulation were evaluated and compared with levels in the remaining patients. No significant differences were found, suggesting that maternal-fetal bonding in prenatal diagnosis patients is not influenced by the indication for the procedure in spite of possible differences in preresult anxiety levels.

The influence of ultrasonography on maternal attitudes toward the fetus were significant, with patients reporting that seeing the fetus on a sonogram made them feel less anxious about the fetus but more anxious about the possibility of an abnormality; overall the ultrasonography engendered positive feelings toward the pregnancy. It is likely that the ultrasonographic examination performed immediately before the preresult bonding evaluation may have contributed to the significant bonding levels found at the time of the procedure.

We conclude that in patients undergoing prenatal genetic testing, maternal-fetal bonding can be demonstrated as early as 10 weeks' gestation and increases significantly once test results are known to be normal. Seeing the fetus on ultrasonography and being informed of normal results were important factors influencing the development of this bonding. The increase in bonding occurred approximately 5 weeks earlier in patients undergoing CVS compared with those undergoing amniocentesis, suggesting CVS may be preferable to amniocentesis with respect to the process of maternal-fetal bonding.

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The Pathology of Maternal Mortality

- 1.0 Introduction
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 - 1.3 Sample selection
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 - 7.4 Complex pathogenesis
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 - 7.7 Infection or sepsis
 - 7.8 Sicklemic-thalassemic manifestations
 - 7.9 Status asthmaticus
 - 7.10 Thrombotic thrombocytopenic purpura
 - 7.11 Thyrotoxicosis
 - 7.12 Toxic pancytopenia
 - 7.13 Unclassified
- 8.0 Pathologic investigation of maternal deaths
- 9.0 References

The pathology of maternal mortality

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1.0 Introduction

1.1 Scope

Properly construed, the pathology of maternal mortality links the concepts of (1) gestational pathophysiology and (2) pathogenesis of lethal lesions. In this context, "lesion" is to be read as ultrastructural and chemical derangement, as well as those histologic changes in tissue traditionally examined and classified by anatomic pathologists. We anticipate that in time better tools will become available to study these matters at fundamental levels of life function. For purposes of this discussion we will describe in some detail those pathologic conditions in our recent experience that caused maternal death in Massachusetts during the 20-year period 1966. to 1985 as representative of current epidemiologic and clinical concerns. This is part of a series of about 1100 maternal deaths that will be reported in further detail elsewhere. We add other cases to illustrate the range of lesions possible under various conditions. The variation of lesions will be emphasized. Maternal death, as a matter of general public health interest, touches numerous other aspects of medicine, obstetrics, and pa-

Mammalian pregnancy, a natural and healthy state, represents the culmination of multiple adaptations of

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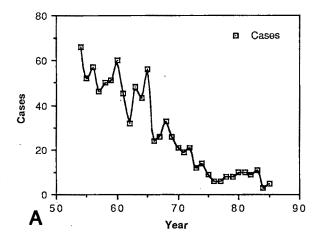
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structure and function. Either or both may predispose to lesion formation. Some of these are unique to pregnancy; others are not, with a varied influence of pregnancy. There are three degrees of gestational influence in the latter group: The first is those conditions that although intercurrent, are enhanced by pregnancy. The second is a group in which pregnancy is a minor or uncertain factor in progression or prognosis. The third is those fully intercurrent, occurring on the same apparently random basis in pregnant women as in the population at large without evidence of mutually adverse effects on pregnancy from the maternal point of view.

Nevertheless, our experience in reviewing this series of 281 maternal deaths suggests that some cases cannot be classified in such a straightforward fashion. The clinical attributes of each topic in the classification scheme will be developed as a composite to the extent possible. The reader should keep in mind that these profiles are not exclusive statements of possible signs or symptoms. As a further contribution to this understanding, we include brief abstracts of one or more case histories with each of the major categories. These abstracts illustrate both specific features of individual cases and provide diagnostic criteria for assignment of cause.

1.2 Historical overview

The current era of professional and public concern over maternal mortality must be considered to have begun with the reported work of Semmelweis. 42 This historical perspective is at root an exercise in pragmatism in that his preventive modality for puerperal sepsis antedated by a generation a proof of the germ theory of certain infectious diseases,18 including puerperal sepsis itself. Subsequently, further general improvement in the maternal mortality rate followed specific developments in anesthesia, blood banking, antimicrobials, and systematized prenatal care.32,40 However, in the opinion of the senior author, the epitome of this approach became manifest when it was integrated with evolving concepts of clinical pathophysiology exemplified by the leadership in Massachusetts of the late Dr. John Figgis Jewett. When the working party (Shanklin and Sommers) sought access to the Massachusetts Maternal Welfare Committee records



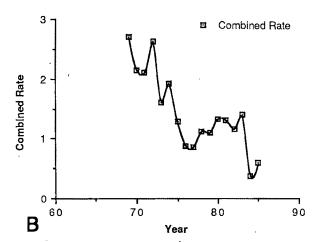


Fig. 1. Number and rates of maternal mortality. A, Graphic depiction of number of maternal deaths in Massachusetts, 1954 to 1985. Note sharp decline in absolute number of cases from 1965 (56 cases) to 1966 (24 cases). B, Graphic depiction of rates of maternal deaths in Massachusetts, 1969 to 1985. Note further decline in rate with the additional reduction in absolute numbers in the equivalent section of part A. Combined rate is maternal deaths per 10,000 live births plus still births (total parity). The rates for 1969 to 1974 ranged from 1.597 to 2.7 per 10,000; the rates for 1975 to 1985 ranged from 0.371 to 1.277 per 10,000.

for this survey, Dr. Jewett gladly approved it. Sadly, just a few months after we began the review, John Jewett died.

1.3 Sample selection

As we worked our way deeper into the records, it became clear that a modern period of 20 years (1966 to 1985) existed that qualified for special emphasis on the following grounds:

A. A charting of the numbers of cases occurring per year in the Commonwealth over the interval 1948 to 1985 revealed a clear and gratifying decline between 1964-65 and 1966-67, against little change in total

annual births, with a further reduction since then (Fig. 1).

- B. As the most recent period, it was considered to more effectively represent those diseases and disorders likely to occur in the population in the near future.
- C. As the most recent period critically examined by both the committee and the working party, it had a better chance of relevance to current methods of diagnosis and treatment.
- D. The period still had 281 maternal deaths, a sample far in excess of what any given practice or hospital might have available for study.
- E. By the 1960s the Committee had become accepted and institutional cooperation in the provision of information and documentation to the field investigators was at a high level. This salutary state of affairs was due to the efforts of Dr. Jewett and the Committee, much enhanced through publication of selected case reports in the *New England Journal of Medicine* carefully, critically, and fairly analyzed.

1.4 Study methods

The residuary files were systematically reexamined year by year by the working party, and data records were put into a prepared microcomputer file scheme. Remnant information by which individual persons could be identified was omitted from the computer data base. After preliminary classification by various obstetric concerns (e.g., section versus vaginal birth, labor management, anesthetic use, pregnancy age, puerperal survival), the cases were rearranged by gross clinicopathologic criteria into arenas to be described.

2.0 Working definition of maternal mortality

2.1 Definition developed from this study

The working party proceeded independent of any particular definition of what constitutes a maternal death, preferring to let the accumulated experience speak for itself. The committee had quite obviously used a comprehensive, inclusive definition and was assiduous in ferreting out cases and information.⁴⁰

During this preliminary assessment of the data, it became apparent that 120 days postpartum would be the most directly useful upper cutoff time interval other than for gestational trophoblastic disease. As will be developed, some pregnancy-related conditions did not prove to be lethal until later, but as a practical procedural definition, maternal death is one in which a woman dies while bearing or just relieved of conceptual tissues or dies up to 120 days after the passage of placental sac, embryo, fetus, or child. It will be seen that this definition includes women dying of chorioma or choriocarcinoma inclusive of complications of their treatment over time. More recently, Allen, Chavkin, and Marinoff¹ used 6 months as a time-specific puerperal interval for inclusion as a maternal death.

Table I. Perinatal mortality in 281 cases of maternal mortality

Conceptual sacs		285*
Ectopic pregnancies	9	
Maternal deaths related to early pregnancy termination	4	
Presumptively viable fetus dying with mother	49	
Subtotal, antenatal maternal deaths		62
No embryo (choriomatous neo- plasm)		6
Less case adjustment subtotal		$\overline{68}$
Puerperal maternal deaths	(net)	217
Fetus stillborn	52	(23.96%, of 217)
Neonatal death	19	(8.76%)
Neonatal survival	145	(66.82%)
Outcome unknown	1	(

^{*}Includes two sets of twins and one of triplets; one twin gestation (Miniabstract 14) resulted in a living infant and a fetus dying with the mother. The latter has been ignored for purposes of determining the perinatal death rate among puerperal maternal deaths.

2.2 The American College working definition

This should be compared with the current form of the consensus statement provided by the American College of Obstetricians and Gynecologists (ACOG) in cooperation with a number of other interested organizations under the title: "Standard terminology for reporting of reproductive health statistics in the United States2:"

Maternal death: The death of a woman from any cause related to or aggravated by pregnancy or its management (regardless of duration or site of pregnancy) but not from accidental or incidental causes.

Direct obstetric death: The death of a woman resulting from obstetric complications of pregnancy, labor, or the puerperium; from interventions, omissions, or treatment; or from a chain of events resulting from any one of these.

Indirect obstetric death: The death of a woman resulting from a previously existing disease or a disease that developed during pregnancy, labor, or the puerperium that was not due to direct obstetric causes, although the physiologic effects of pregnancy were partially responsible for the death.

The ACOG statement went on to limit indirect obstetric death in the following note: "Death occurring to a woman during pregnancy or after its termination from causes not related to the pregnancy nor to its complications or management is not to be considered a maternal death. Nonmaternal causes may result from accidental events (e.g., auto accident or gunshot wound) or incidental causes (e.g., concurrent malignancy).2"

The consideration that went into the ACOG definition notwithstanding, the working party came to a different perspective. The influence of pregnancy on intercurrent disorders and vice versa may be subtle or simply not yet known. Accordingly, we propose that all deaths closely related in time to pregnancy be included as the point of origin of concern and investigation. Dr. Jewett's committee very clearly used essentially this approach and for good reason. After close review, the working party concluded it could not dismiss accidents or homicides as maternal deaths because psychologic factors derived from pregnancy or puerperium may have been significant contributory or even proximate causes of the deaths just as much as physiologic factors or specific diseases might be in others. This point will be further considered later.

3.0 Perinatal mortality within maternal mortality

3.1 Net sample size

These 281 mothers carried 285 conceptual sacs. The excess is due to two sets of twins and one set of triplets. Consideration of true perinatal mortality under these circumstances requires an adjustment to find the number of fetuses and newborns at independent risk born before maternal death and several other aspects of the matter. This is shown in the upper part of Table I. Note that the overall fetal loss from women dying antenatally was a substantial 16.9% (48 of 284). The rest of the difference is accounted for by (1) lack of embryo (choriomatous neoplasm), (2) ectopic pregnancy, or (3) maternal death related to pregnancy termination. There was one case lacking fetal outcome.

3.2 Perinatal mortality

The overall perinatal mortality rate was 32.7%, of which about three quarters were stillborn (23.9%) and one quarter were neonatal deaths (8.8%). The data are provided for comparison with the perinatal outcome in the various subcategories of maternal death discussed below.

4.0 Classification of maternal mortality

4.1 Arrangement of the classification

The entire series of 281 maternal deaths was closely examined for the appearance of related points in history, course, outcome, and autopsy findings. These led in all but one case to a summary statement of the central pathophysiologic theme of each case. During the summarizing assembly of these themes, it became apparent that significant supportive themes or findings were often present that warranted inclusion as secondary points in the classification to be presented. Sections 4.2, 4.3, and 4.4 contain the three primary groups in the order noted above (Section 1.1) listed by primary and secondary attributes. In some instances a combination of secondary attributes is given. Numbers in parentheses indicate when more than one case fits the combined primary and secondary criteria.

- 4.2 Group one: Conditions unique to pregnancy (83 cases; 29.54%)
 - 4.2.1 Amniotic fluid embolism/disseminated intravascular coagulation (AFE)/(DIC) (25 cases)

Abruptio placentae

Cervical lacerations

During episiotomy repair

During section

Early puerperal

Late labor

Late puerperal

Not in labor (2)

Occult uterine rupture

Oxytocin overdose (8)

Oxytocin overdose, uterine rupture

Probable, puerperal (2)

Tumultuous labor (3)

Uterine tear or rupture

4.2.2 Ectopic pregnancy (9 cases)

Abdominal pregnancy

After adnexectomy

Cornual pregnancy (2)

Ruptured tubal (5)

4.2.3 Gestosis (24 cases)

Acute fatty liver

Basilar artery aneurysm

Eclampsia (11)

Hepatic necrosis, massive peritoneal hemorrhage (2)

Puerperal coma without apparent seizures (2)

Intracranial hemorrhage (2)

Late puerperal (2)

Magnesium sulfate overdose

Myocardial infarction

Thiazide misadventure

4.2.4 Hemorrhage, nongestotic, extracranial (12 cases) Abruption/DIC Abruption, hyperhydration

Abruption, ruptured uterus

Atony, probable

Lacerations, vagina

Laceration, vagina, occult gestosis

Previa/accreta; cervical laceration;

oxytocin

Shoulder dystocia; lacerations, cervix, vagina, subserosal uterus

Uterine rupture (2)

Uterine rupture, osteogenesis imperfecta

Uterine rupture, after oxytocin

4.2.5 Neoplasms, obstetric (6 cases)

Choriocarcinoma (5)

Chorioma

4.2.6 Therapeutic misadventure (7 cases)

Cardiac arrest during examination under anesthesia

Epinephrine overdose at cervical conization

Methylergonovine maleate overdose

Phenytoin withdrawal, acute

Saline abortion (3)

- 4.3 Group two: Pregnancy probable major contributory factor (54 cases, 19.22%)
 - 4.3.1 Air embolism (1 case)

Intracoital

4.3.2 Anesthetic misadventure (13 cases)

Analgesia/anesthesia for abortion Aspiration pneumonia (2) Aspiration, self-extubation, gestosis Cardiac arrest, difficult entubation

(2)

Gas embolization

Hysterectomy, hemorrhage (?AFE)

Massive aspiration (2)

Spinal overdose (2)

Spinal overdose, congenital heart disease

4.3.3 Cardiovascular factors—congenital

heart disease (7 cases)

Atresia, aortic arch

Atrial septal defect

Barlow syndrome (prolapsed mitral valve)

Eisenmenger syndrome (2)

Ventricular septal defect (2)

4.3.4 Pulmonary thromboembolism (15

After hysterectomy

After hysterotomy

After laparotomy

After ovarian cystectomy

Carcinoma, rectosigmoid

Hemangiosarcoma

After tubal ligation Rheumatic heart disease post com-Early puerperal (2) missurotomy Late puerperal (4) Thrombotic occlusion, internal ca-Thrombophlebitis, femoral (2) rotid artery Thrombophlebitis, pelvic and ovar-4.4.3 Complex pathogenesis (17 cases) ian veins Acute yellow atrophy, liver, Alzheimer type 2 glia Thrombophlebitis, plus renal an-After epileptic state giomyolipoma 4.3.5 Renal failure (2 cases) Cardiomyopathy, scleroderma End-stage nephrosclerosis (2) lung, pulmonary hypertension Sepsis/infection (16 cases, 30.8% of Diabetes mellitus, brain stem inall sepsis/infection cases) farction, tricuspid endocarditis Accreta; Aerobacter Diabetes mellitus, cardiac arrest After intrauterine contraceptive Ectopic pregnancy, hyperhydration, hypophosphatemia device (IUD) ?Fanconi syndrome, ?polyadenoma After IUD, septic myocardial infarctions syndrome Postabortal, clostridial (2) Hepatitis, possible drug abuse Hepatorenal syndrome Probable, late puerperal (2) Lupus nephritis, poststeroidal ad-Puerperal sepsis (streptococcal) (7) renal atrophy Puerperal sepsis, gestosis Rectovaginal septal hematoma, sec-Paroxysmal nocturnal hemoglobinondary ligation uria; peptic ulcer, hemigastrec-4.4 Group three: Intercurrent disease or pregtomy, cholecystectomy nancy an uncertain or minor factor (144 Pulmonary thromboembolism, cases, 51.25%) ruptured caudate lobe of liver 4.4.1 Accidental (24 cases) (?gestosis) Ruptured aneurysm, splenic artery; Aspiration from unknown cause lupoid hepatitis Diazepam overdose Drowning, epileptic state Sarcoidosis, systemic (2) Gas explosion Status appendectomy, adult respi-Homicide (5) ratory distress syndrome Wegener's granulomatosis Intravenous drug overdose, pre-4.4.4 Intracranial vascular incident, nonsumed heroin gestotic (26 cases) Physical fall (stairs) (2) Physical fall (second story window, Arteriovenous malformation (5) house fire) Brain stem infarction Probable suicide Paradoxic embolism Propoxyphene overdose Presumed aneurysm (3) Salicylate toxicity Presumed late puerperal aneurysm Vehicular accident (7) Vehicular accident, delayed mani-Presumed thrombosis (2) festations Proven aneurysm (6) 4.4.2 Cardiovascular factors—acquired Proven aneurysm, congenital heart heart disease (13 cases) After mitral replacement Proven aneurysm, oxytocin Arteriosclerotic cardiovascular overdose disease Superior sinus thrombosis Thrombosis, middle cerebral artery Cardiomyopathy (2) Coronary dissection 4.4.5 Neoplasm—nonobstetric (16 cases) Kyphoscoliotic heart disease Adenocarcinoma, bowel, unspec-Marfan syndrome; dissecting aortic ified hematoma Astrocytoma (4)

> Myocardial infarction (3) Rheumatic heart disease

Table II. Group one: Maternal deaths from conditions unique to pregnancy

Category of disorder	No. of cases	% of whole series
Amniotic fluid embolism/dissem- nated intravascular co- agulation	25	8.897
Ectopic pregnancy	9	3.203
Gestosis (toxemia)	24	8.541
Hemorrhage, nongestotic, extra- cranial	14	4.982
Neoplasm, obstetric	6	2.135
Therapeutic misadventure	7	2.491
GROUP TOTAL	85	30.25

Hodgkin's lymphoma

Melanoma

Meningioma

Mammary carcinoma (2)

Oat cell carcinoma, lung

Pheochromocytoma (2)

Thymoma, lymphoblastic leukemia

4.4.6 Sepsis/infection (36 cases; 69.2% of all sepsis/infection cases)

Bacterial endocarditis

Endotoxemic (3)

Hepatitis (3)

Herpes encephalitis

Influenzal pneumonia (3)

Influenzal pneumonia, hyperten-

sive heart disease

Intestinal obstruction (2)

Intrapericardial vena caval rup-

ture, septic

Klebsiella

Meningitis, cerebritis

Meningococcemia

Mixed bacterial-viral pneumonia

Necrotizing pneumonia

Peritonitis (4)

Pneumococcal pneumonia

Pneumococcal pneumonia, menin-

gitis

Pneumonia, postoperative

Pneumonia, unspecified

Streptococcal meningitis

Ulcerative colitis

Varicella myocarditis

Viral pneumonia (2)

4.4.7 Sicklemic/thalassemic manifestations (3 cases)

Cerebral thrombosis

Crisis, thalassemia (2)

4.4.8 Status asthmaticus (2 cases)

Delayed puerperal

Late gestational

4.4.9 Thrombotic thrombocytopenic purpura (4 cases)

Cerebral infarction, intraventricu-

lar hemorrhage

Intracranial hemorrhage

Lupus nephritis

Subcapsular hematoma, liver

4.4.10 Thyrotoxicosis (1 case)

Possible added hepatitis

4.4.11 Toxic pancytopenia (1 case)

Folate deficiency, nitrous oxide effect

4.4.12 Unclassified (1 case)

Unclassified

5.0 Mortal conditions unique to human gestation

5.1 General

This group accounted for 83 of the 281 cases or 29.54% of all maternal deaths for the 20-year period. Assignment here was on the reasonable basis that gestational changes in the reproductive tract; the presence of conceptual products; and clinical efforts at diagnosis, management, or treatment were and are, by self-definition, unique to pregnancy. Thus disseminated intravascular coagulation, a difficult diagnostic syndrome induced by many pathologic processes, is best classified here through its link to amniotic fluid infusion of embolism. The task then, for that condition as an example, becomes one of understanding the variety of pathogenetic mechanisms by which the normally salutary physical and chemical buffering effects of amniotic fluid are cast aside and disaster strikes.

The mortal conditions related to abnormal implantation are obvious: Ectopic pregnancy remains difficult to identify and manage, abruptions still occur, and placenta previa with or without accreta has not been eliminated. The questions of causality of toxemia, better known in Europe as gestosis, are less rancorous with newer insights on the role of nutrition, metabolic injury, and atypical renovascular responses to pregnancy, 19, 46 but severe forms of the disorder remain a major factor in maternal death. 10 Despite generally effective che-

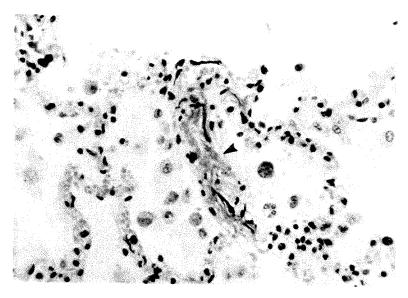


Fig. 2. Amniotic fluid embolization. Peripheral lung parenchyma showing elongate cluster of transported squamous cells and a small, recent segmental thrombosis (arrow). (Hematoxylin and eosin; original magnification ×250.)

motherapy against chorionic malignancy, the problem has not gone away. Finally, treatment or medication during pregnancy and labor remain occasional sources of serious injury and, sometimes, death. The relative frequencies of these subgroups are shown in Table II.

5.2 Amniotic fluid embolism (25 cases)

This process is clearly far more common than is appreciated in the obstetric literature.49 The hypervascularity of the uterus and other pelvic structures in pregnancy and the considerable pressures that labor can generate make such a risk one easily understood a priori. However, two fatal cases occurred in women who were not in labor at all.

It should be noted that some significant distinctions exist in time between infusion of amniotic fluid into the maternal circulation, the onset of clinically observed or significant symptoms, and the time of death thereafter. No strict pattern of manifestation is seen, but several typical expressions of the disorder are seen.

A profile of the woman with amniotic fluid embolism/infusion follows: She is usually 25 years of age or older, in her third, fourth, or fifth pregnancy; and is at or very near to term (37 to 41 weeks' menstrual age). She is more likely not to have sustained a prior fetal loss or miscarriage. This combination of age and parity is occasionally replaced by a young primigravida with precipitate labor or tetanic contractions. Although about 8% of cases occur before labor begins, vigorous labor is the rule. This can be spontaneous or related to use of uterine stimulants. In fact, almost half occur during or after oxytocin/tocosamine overdose. The second most important factor is a tear somewhere in the birth canal, affording access for amniotic fluid into

the maternal venous system. Fetal malpresentation contributes to amniotic fluid infusion. For example, in one patient a hydrocephalic fetus could not enter the pelvis during labor.

The herald of the infusion is usually sudden and may be clinically silent, with later onset of disseminated intravascular coagulation as the first clear sign. The sign can be apnea, the appearance of embolism in the chest, tachycardia, or sudden onset of fever. In several instances a seizure was the first sign, attributed to pulmonary insufficiency and acute hypoxia from massive lung embolization.

The onset is concentrated about the immediate perinatal time frame, in particular from about 1 hour before delivery to 30 minutes afterward. The final few minutes of labor carry as much risk as the immediate postnatal few minutes. The sudden changes in intrapelvic pressure and blood flow that occur with fetal passage seem to enhance amniotic fluid infusion.

The embolization can be so massive that survival thereafter is measured in minutes. Eight of 25 women died in the first 6 hours postpartum, and 72% died on the first day. This concentration of survival weakens attribution of late deaths to amniotic fluid embolization. In later deaths the intermediate steps of disseminated intravascular coagulation and hemorrhage must be documented. A unique case showing both amniotic fluid embolism and delayed pulmonary thromboembolism is presented below in abstract form.

The pulmonary lesions of amniotic fluid embolization consist of amniotic fluid bubbles and transported fetal squamous cells regularly found in amniotic fluid, which are commonly seen in air spaces of fetal or neo1134 Shanklin et al. October 1991
Am J Obstet Gynecol

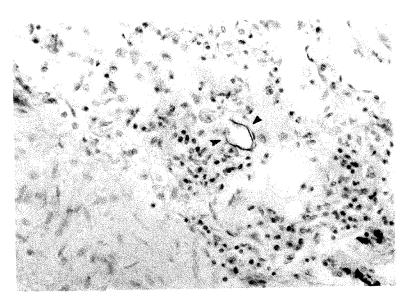


Fig. 3. Amniotic fluid embolization. Nonstaining intravascular bubble of amniotic fluid demarcated by surface-adherent embolic fetal squamous cells (arrows). The photograph is necessarily limited to a single focal plane; focusing up and down over such foci confirms the molded spheroidal form expected from a fluid bubble or droplet with a surface tension interface within lung vessels. Air or nitrogen embolism has a similar appearance. (Hematoxylin and eosin; original magnification $\times 250$.)

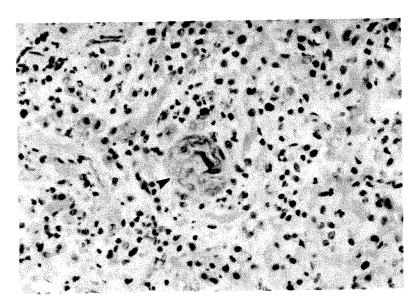


Fig. 4. Amniotic fluid embolization. Retained and intact clump of embolic squamous cells within a field of subacute pneumonia. Thirty-three-year-old gravida 3, para 2, dying 22 days postpartum. Multiple foci of embolic squames and some lanugo hair were also present. (Hematoxylin and eosin; original magnification $\times 250$.)

natal lungs after various late pregnancy stresses. These cells are easier to find (Fig. 2), but bubbles can be identified as spheroidal fluid-filled spaces within lung capillaries, venules, and arterioles. Their shape can be confirmed by skillful focusing at multiple planes during microscopic examination. Occasionally one is rewarded by finding squames and bubbles together (Fig. 3). The

embolic squames are resistant to digestion, even when pneumonitic exudate is present (Fig. 4). Their persistence is helpful in recognizing amniotic fluid embolization beyond the more common time period for this disorder (see Miniabstract 1, Section 5.2.1).

Glomerular lesions are important in the assessment of amniotic fluid embolism or subsequent disseminated

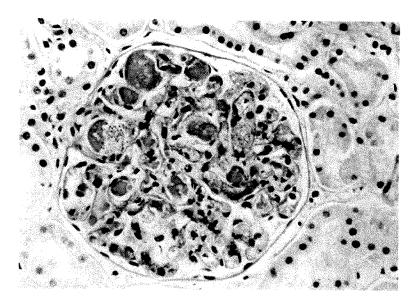


Fig. 5. Amniotic fluid embolism; disseminated intravascular coagulation. Multisegmental glomerular thrombosis in left kidney. Some granular formalin pigment is present. Tumultuous labor in a 45year-old gravida 6, para 5; death 7 hours postpartum. Right kidney had been removed 13 years earlier for hypernephroma. Left kidney also contained a small angiolipoma. (Hematoxylin and eosin; original magnification $\times 250$.)

intravascular coagulation. Fig. 5 shows multiple lobular thrombi within capillaries of the glomerular tufts. The working party did not find squames secondarily transported to glomeruli. This theoretic possibility might occur because of intrapulmonary vascular shunts after segmental obstruction. By contrast, fluid bubbles were seen in a few cases and should be sought in all deaths close in time to the ictal event.

Amniotic fluid infusion is a significant cause of perinatal death as well. In this series two fetuses died with the mother, six were stillborn, and one died 2 hours postnatally. This perinatal mortality rate of 36.3% is essentially the same as the overall rate of the 20-year series (Table I), with a closely similar distribution between stillbirth and neonatal death.

5.2.1Miniabstract 1

A 22-year-old woman, gravida 3, para 1, abortus 1, had her first pregnancy at term 2 years earlier, with a birth weight of 3200 gm. In the forty-first week labor was augmented by 5 IU oxytocin/500 ml in 5% dextrose in water at an unknown rate. Labor was noted to be vigorous and almost continuous. Artificial rupture of the membranes (AROM) occurred just before delivery of a 3600 gm surviving newborn. Two hours postpartum, there was a sudden onset of marked bleeding. Two units of O- and two units B-type specific blood were given. A hysterectomy was performed 5 hours postpartum. On the eighth day massive pulmonary thromboembolism (PTE) occurred, and the patient died. Autopsy showed renal cortical necrosis and nonorganizing recent thrombi in ovarian and renal veins and the inferior vena cava. Microscopically, widespread AFE was also found.

Comment: This combination of AFE and PTE is rare. In fact, this was the only proven case in the entire series

of more than 1100 cases. A direct pathogenetic relationship is unclear. A factor to be considered is that excellent, prompt surgical and general medical response to the early amniotic fluid embolization allowed survival into the risk period for thromboembolism of the ordinary puerperal sort. The possibility of a rebound of coagulation factors into a hypercoagulable state cannot be excluded, albeit in this case daily determinations of the ordinarily studied factors were not done.

5.2.2 Miniabstract 2

A 44-year-old woman, gravida 2, para 1, was scheduled at term for repeat section because of a deformed pelvis presumably caused by poliomyelitis. Current pelvic evaluation suggested no pelvic deformity of obstetric significance. Her weight fluctuated in the late third trimester, from 70 up to 75 and then down to 69.6 kg. In early labor she was given pentobarbital, 100 mg, at 7:55 PM; meperidine, 75 mg, and atropine, 0.4 mg, were administered at 8:05 PM. She developed persistent emesis. with episodes at 8:30 PM and 8:45 PM, bore down at 8:50 PM, and became cyanotic at 8:51 PM. An immediate moribund section was done. The mother died 20 minutes postpartum. The 3629 gm slowly responsive infant died at 14 hours. Autopsy on the mother revealed pulmonary amniotic fluid embolism and a large crumpled sheet of amniotic membranes within the right pulmonary artery.

Comment: Pathologists with experience in posttraumatic injuries look for embolism of tissues such as bone marrow, splenic pulp, and brain. This is merely the pregnancy extension of this general principle: bulk deportation of tissue fragments. The ready deformability of the membranous amniochorion might more often

Table III. Maternal deaths from gestosis ("toxemia")-related conditions

Category of gestosis/colesion	No. of cases	% of whole series
Acute fatty liver	1	0.356
Basilar artery aneurysm	1	0.356
Eclampsia	11	3.915
Hepatic necrosis, massive peritoneal hemorrhage	2	0.712
Intermediate puerperal	1	0.356
Intracranial hemorrhage, not otherwise specified	2	0.712
Late puerperal	3	1.068
Magnesium sulfate overdose	1	0.356
Myocardial infarction	1	0.356
Thiazide misadventure	1	0.356
GROUP TOTAL	24	8.54

yield this obstetric disaster were it not for the limited size range of the inner uterine venous sinuses in late labor and early puerperium.

These two cases illustrate extremes in the lesional potential of embolism of amniotic content, one hidden from clinical and pathologic view except through the possibly connected sequel of pulmonary thromboembolism, and the other a mass transport of portions of the reflected membranes. Although uncommon, both require autopsy to confirm or rule out. The rule of thumb would be that massive embolism is fatal or severely distressing very close to delivery; lesser degrees manifest later; and as suggested by the first case, death or severe distress may occur in the time frame more likley attributed to thromboembolism not derived from the effects of amniotic fluid infusion.

5.3 Ectopic pregnancy (9 cases)

On a priori grounds abdominal ectopic pregnancy would more likely manifest in later pregnancy. The one case represented here is a partial exception, with death occurring at an estimated 14 to 16 weeks' menstrual age. Nevertheless, such a view is confirmed by this small series, although at an interval much less than with some reported abdominal pregnancies. The other eight cases had a range of gestational age of 5 to 11 weeks; two were themselves only estimated ranges from reconstructed menstrual histories, 8 to 10 and 8 to 11 weeks, respectively.

The pathologic findings are those of disruptive hemorrhage in the uterine tube or cornu with or without extrusion of the conceptual sac. The implantation may be on the opposite side of the adnexae from the corpus luteum of pregnancy. The problems of interpretation of signs and symptoms of ectopic pregnancy can be mitigated by approaching all examples of early pregnancy distress as potentially those from ectopic implantation.

5.3.1 Miniabstract 3

The patient was 35 years old. Her parity was unrecorded. She had severe pelvic pain at first with eventual physical col-

lapse. Multiple telephone calls were made to physicians to attend her at home without success, and she steadfastly refused to report to an emergency room (ER) or to be taken there by her husband for 6 hours. She apparently became unconscious about 3 hours before actually visiting an ER at 11:55 pm. Fifteen minutes after arrival at the ER she went into cardiac arrest; vigorous resuscitation for 40 minutes was unsuccessful. Autopsy revealed a very obese woman, greater than 111 kg (250 pounds) and 162 cm (64 inches) tall. A ruptured tubal ectopic site at an estimated 5 weeks' gestational age was found. About 1500 ml of blood filled the abdomen. An unruptured 900 gm simple serous cyst of the right ovary was also present.

5.3.2 Miniabstract 4

A 26-year-old woman, most likley a primigravida, came to the United States as a military bride. She spoke no English. Her soldier husband was on extended duty in the country of her origin. She lived with a man calling himself an "uncle" but who was apparently from the same military unit. He did not speak her language. There were two visits to an ER during the evening for vague discomfort. She was accompanied by this man. There were indications she experienced overt abdominal pain at about 5:30 AM. On the third ER visit 3 hours later (the "uncle" brought her in), she was dead on arrival. Autopsy revealed ruptured tubal ectopic pregnancy and massive abdominal hemorrhage.

Comment: The rapidity of progression of hemorrhage from pelvic vessels dilated by the hormonal effects of pregnancy is impressive. The pathophysiologic conditions do not allow for ceremony in obtaining or giving appropriate obstetric care. Rapid serum and urine tests for chorionic gonadotropin are generally available and highly sensitive. Thus a rupturing tubal gestation should be susceptible to diagnosis. A completed tubal abortion is, fortunately, not usually associated with the type of bleeding ordinarily seen in rupturing ectopic pregnancies. Lest one conclude that the risk for maternal death from ectopic pregnancy is diminishing, one of the nine cases is from the next to last year of this survey (1984).

5.4 Gestosis ("toxemia of pregnancy") (24 cases) This pathophysiologic syndrome continues to be of great clinical significance with the same broad range of

lesions and functional effects that the more classic reports so well delineated.^{15, 28, 54} Table III reflects the overall principal types and our assignment of these to major categories. Several other cases in which some evidence suggested the presence of or the early onset of gestosis were found, but on further analysis this was considered of minor importance in the mortal outcome. Given the occasional cases of puerperal eclampsia without apparent prior warning and reassignment of maternal deaths to gestosis after careful autopsy examination, the possibility of a subtle but nonetheless important factor cannot be readily dismissed. As an example of this, attention is here drawn to the discussion on the relationship between pregnancy and intracranial arterial aneurysms (Section 7.5).

The two dozen mothers with fatal gestosis showed considerable variation in age, gravidity, parity, and gestational age at time of delivery. A few general statements can be made.

5.4.1 Age range

The ages ranged from 15 to 42 years. The mean was 28.4 years, with 28.5 years as the effective median. Although the younger women had generally lower parity as expected, there was one primigravida at age 34 years; the highest gravidity in the younger women was 3 at age 25 years. The age spread is fairly uniform. In fact, the largest cluster is a quarter of the cases at ages 33 to 35 years.

5.4.2 Gravidity-parity

By contrast, gravidity-parity status was less uniform. Contrary to the still common misconception of gestosis as a disorder of first pregnancy, fatal gestosis in primigravidas accounted for only 11 of these 24 women (45.8%). There were five secundigravidas, four at gravida 3, two at gravida 5, and one each at gravida 8 and 9. The average gravidity was 2.5, but the average parity was a low 1.17. The difference was accounted for by both spontaneous miscarriages and pregnancy terminations. The highest parity was 6.

5.4.3 Gestational age

Gestational age at time of delivery seemed to reflect the well-known tendency toward premature onset of labor. Twelve deaths occurred at or near term (38 to 42 weeks inclusive), half the total. The remainder spanned 24 to 37 weeks, with one quarter of the cases clustered at 321/2 to 35 weeks. However, this aspect of gestosis as a factor in maternal death must be examined in the light of the high section/hysterotomy rate, 10 of 24 (41.7%). Seven sections were done before onset of labor and an eighth was done in very early labor when no progress in station or dilation had been observed. These account for 6 of the 12 "preterm" deliveries; 2 were because the mothers died undelivered at 321/2 and 34 weeks. Thus in this experience, early delivery of the fetus was largely, if not wholly, a consequence of obstetric intervention for abruption; eclamptic seizures; or severe, progressive gestosis.

5.4.4 Perinatal mortality

The perinatal mortality is of interest. The rate of fetuses dying undelivered with the mother was half that of the overall series, 2 of 24, 8.3% (against 40 of 281 [17.1%]; see Table I). The stillbirth rate was three quarters that of the whole series, 4 of 22, 18.2% (against 52of 217 [23.6%]), and the neonatal death rate was 2 of 22 (9.1%), essentially the same as the overall rate of 8.7% (Table I). Sections were productive of one stillborn and two neonatal deaths, the former a relatively low figure and the latter relatively high. They are connected in that prompt section to salvage the infant or to interrupt the gestotic process will deliver a number of fetuses already so badly stressed that they do not survive postnatally. The overall survival rate for newborns is a mildly enhanced 72.7% against the overall datum for the series of 66.8% (Table I), but section did not contribute to this improvement. One was delivered at 30 weeks from a primigravida aged 21 years with a prior history of childhood nephritis, a blood pressure surge to 290/180, and three seizures during the confinement. The infant was small for gestational age, had a 1-minute Apgar score of 2, and lived almost a day. The second postsection neonatal death was from a 24year-old primigravida at 241/2 weeks' gestation. There was fulminant eclampsia with a blood pressure surge to 220/120, several seizures, and massive cerebral hemorrhage 3 hours postpartum. The infant weighed about 600 gm and lived almost a day. The sole stillbirth by section is similar. This was a 32-year-old woman, gravida 3, para 2, in the twenty-seventh week of pregnancy. Prenatal care had just begun but was a caloric restrictive, low-sodium regimen. She was in an early obtunded state, and hysterotomy was done for fetal death to interrupt the gestotic process. She survived this by 51 hours; autopsy showed classic eclamptic changes in kidney and brain.

Thus section cannot be independently faulted because all three mothers had fairly severe early gestosis, but because the only two neonatal deaths among gestotic women were section delivered, a positive contribution of section to neonatal outcome is marginal at best and more likely irrelevant. From this experience, it would seem that abdominal section should be done on women with progressive gestosis for obstetric but not for fetal indications.

5.4.5 Pathologic findings

Gestosis, or eclamptogenic toxemia of pregnancy, is a pathophysiologic state with moderately varied pathologic organ findings. Two visceral lesions are especially associated. The first is in the renal glomerulus (Fig. 6) and is essentially a capillary endotheliosis with a more varied mesangial component.29 The second is liver dam-

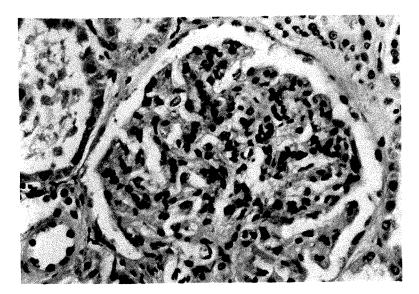


Fig. 6. Glomerulus in severe preeclamptic gestosis; 17-year-old primigravida. Lesion at this power of resolution by light microscopy appears as moderate hypercellularity, some of which is endothelial and some mesangial. Ruptured subcapsular liver hematoma and peritoneal exsanguination were found at autopsy. (Hematoxylin and eosin; original magnification \times 250.)

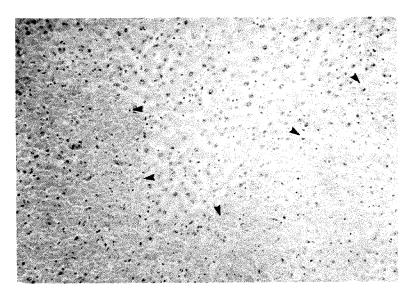


Fig. 7. Liver in severe eclamptic gestosis. Confluent necrosis on three sides of remnant liver tissue showing nutritional atrophy, loss of glycogen. This necrosis is more advanced than that in Fig. 8. Arrows denote a fairly sharp line of demarcation between the necrotic and viable zones. Depending on the degree of added heart failure, sinusoidal congestion will become hemorrhage in the zones of necrosis. (Hematoxylin and eosin; original magnification $\times 100$.)

age. In its most extreme form this is a confluent periportal-to-midzonal necrosis (the change representing extension of the process) (Figs. 7 and 8). The intermediate lesions are less well known. This is most likely because kidney biopsy has been done much more often than has biopsy of the liver. ^{26, 29} More recent findings still under investigation are uterine boundary zone endothelial changes and injury. ⁴⁸

The earliest cardiovascular pathophysiologic change is blood volume restriction, but after renal sodium retention mechanisms have been invoked,³⁸ congestive heart failure may be superimposed with added injury to the liver. In fact, underlying hepatic dysfunction may be found, possibly derived from protein deficiency.^{19, 46} This is reflected in liver cell changes short of necrosis that then lead to cell death and tissue necrosis with the addition of heart failure. Systemic mitochondrial injury

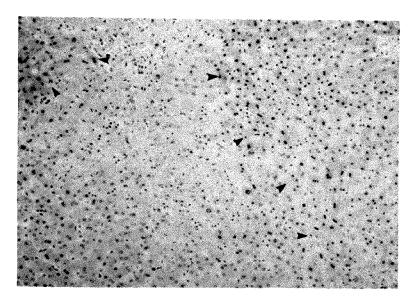


Fig. 8. Liver in severe eclamptic gestosis. Two zones (arrows) of preinfarctive pyknotic cellular changes in midzone of liver lobule. In some cases these changes are even more discrete. State of lesion at autopsy depends in part on the interval of survival and duration and depth of postictal shock. (Hematoxylin and eosin; original magnification $\times 100$.)

Table IV. Maternal deaths from extracranial nongestotic hemorrhage

Abruptio placentae		4
Disseminated intravascular coagulation	2	
Hyperhydration	1	
Ruptured uterus	1	
Atony, uterus		1
Lacerations of birth canal		3
Cervical and vaginal (shoulder dystocia)	1	
Vaginal	1	
Vaginal, occult gestosis	1	
Previa-accreta		1
Rupture, uterus (without abruptio)		5
Isolated	2	
Occult	1	
Osteogenesis imperfecta	Í	
Postoxytocin	1	

is a likely factor here. 48 Blood liver enzyme values rise as one feature of a severe variant of gestosis, the HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), which has resulted in maternal death. 10 The total bilirubin in the cited case report was 4.5 mg/dl, a finding supportive of liver cell injury. Another point in favor of this is a high frequency of diffuse sinusoidal congestion in livers as seen by the working party in review. This in turn relates to capsular hematomas and intraperitoneal rupture and hemorrhage. Because the terminal mechanisms are those of acute hypovolemic anemia, little blood is present to pool passively in the liver sinsuses as part of the agonal events. It more likely that this occasional finding is the remnant of an earlier sequestration from adaptive hemometakinesis compounded by right heart failure.

Table III shows that three maternal deaths were the result of severe liver injury. Acute fatty liver is an advanced metabolic injury^{30, 36} with further consequences of hepatomegaly and interference with protein synthesis including clotting factors. The relation of this to microvesicular fatty change⁷ is currently under study. Although the renal lesions have been better studied, in cases of gestosis in which fatty liver dominates, the lethal lesions are in the brain and not the kidney. Superimposed acute tubular necrosis is unusual.28 Clinical renal shutdown from failing prerenal circulation is more common; if renal failure is deep but transiently relieved, the more advanced lesion may be found later at autopsy.

Depending on treatment modality, lung injury in the form of adult respiratory distress syndrome may be superadded to this list. Diffuse edema of the cerebral neuropil is often found to supplement the glomerular and hepatic changes of gestosis. This does not preclude the occurrence of overt space-occupying intracranial hemorrhage. In some instances hemorrhage is just the effect of rapidly progressive hypertension on a congenital aneurysm. Medial defect aneurysms of the major intracranial arteries are fairly common in women of childbearing age.24,25 Mass hematomas in the lenticulostriate region of the internal capsule are often so destructive of tissue that an underlying aneurysm cannot be identified. The extent of some of the hematomas is shown by one filling the third and both lateral ventricles with extensive subarachnoid hemorrhage. Such hematomas should not preclude close study of the kidney and liver to ascertain whether the underlying disorder is that of gestosis.

Of the lesser associated lesions, the one case of myocardial infarction is especially instructive. This woman had typical moderately severe gestosis without seizures. She was a 23-year-old primigravida with a strong family history of hypercholesterolemia. The blood pressure surged to 200/110 during labor. The delivery was by midforceps using local and nitrous oxide anesthesia. The 2722 gm infant lived. On the eighteenth puerperal day the episiotomy broke down and a secondary repair was necesary. Five days later she died suddenly at home. Autopsy revealed severe coronary arteriosclerosis; occlusion of the left anterior descending coronary artery; diffuse necrosis of an enlarged left ventricle; and diffuse, severe pulmonary edema.

Therapeutic misadventures add to the underlying burden of gestosis. In one magnesium sulfate and hypotensive agent overdose (10 ml, 50% intravenous magnesium sulfate plus 10 ml reserpine [2.5 mg/ml]) was compounded by the use of spinal anesthesia in an obese woman with small stature and short neck (weight, 102 kg; height, 152 cm or 59.8 inches). She died 5½ hours postpartum. An intense 5-week use of thiazides in another patient greatly constricted the blood volume, leading to hypovolemic anemia and hypotension. The pancreas was not examined at autopsy for evidence of thiazide effects.^{4,11}

5.4.6 Miniabstract 5

The patient, a 21-year-old gravida 1, had a small gynecoid pelvis, and was 148.6 cm (58.5 inches) tall. At 9 years of age she was diagnosed as having "nephritis." A questionable past history of epilepsy existed. Her blood pressure rose during pregnancy from 120/86 to 240/160; the urine albumin increased to 4⁺. In the thirtieth week of gestation she complained of headache, was admitted, and her pressure surged to 290/180. There were three seizures. She collapsed and became apneic. Section delivery produced an unweighed "small" newborn with an Apgar score of 2, who died later the same day. The mother died 24 hours after delivery. Autopsy revealed typical gestotic glomerular lesions and no evidence of prior neprhitis. Multilobular he-

patic necroses and numerous intravascular fibrin deposits were found throughout the brain.

Comment: This case was also briefly noted above. It is presented here in greater detail to illustrate that occasionally the clinical history of predisposing renal disease is not confirmed by autopsy study. The childhood records were not available to the committee nor to the working party, but cogent autopsy examination of the kidneys ruled out any remnant or contributory lesion of the kind usually designated as nephritis.

5.4.7 Miniabstract 6

A 34-year-old woman, gravida 2, para 1, delivered in the thirty-third week of pregnancy. The first pregnancy was unremarkable. Her blood pressure rose in early third trimester to 180/108 and then spiked to 190/120 with onset of abdominal pain and headache the day before section delivery. She was treated by three intramuscular doses of magnesium sulfate, 2 gm each in rapid succession, and was then sent to a second hospital with a diagnosis of "sepsis." On arrival there she was obtunded, apparently having sustained a seizure en route. Hydralazine hydrochloride, 20 mg, and more magnesium sulfate, 4 gm, were given rapidly by vein. Shortly thereafter she went into respiratory arrest, and calcium gluconate was given with effective results. Respirations remained spontaneous. Section at this point produced a surviving newborn weighing 1590 gm. The mother continued to have both cerebral and lung difficulties, was treated with barbiturate suppression, and died on the eleventh puerperal day. Autopsy showed typical renal lesions of gestosis, a massive left intraventricular cerebral hemorrhage, and another smaller one in the right basal ganglia. No evidence of sepsis was found.

Comment: This was not coded as a magnesium sulfate misadventure because of (1) the prompt recovery after calcium injection, (2) the massive intracranial hemorrhaging, and (3) the delayed puerperal death. To be considered a magnesium sulfate death, the sequence would have to be closer in time and more direct in process despite the loading doses over a short time period.

5.4.8 Miniabstract 7

The patient was a 41-year-old gravida 6, para 5, at term. All prior pregnancies were uneventful. Her blood pressure had been elevated for 3 weeks and peaked at 200/130. She had marked foot edema and 4⁺ albuminuria. Three days before delivery she was in an automobile accident but had no major injuries; "bumped about" best describes her condition. Labor ensued and was augmented by six doses of oxytocin intramuscularly, 2 minims each. She had a seizure and lapsed into a coma. A Vorhees bag was unsuccessful; version was begun but the cord prolapsed as the mother went into a coma. Nitrousoxygen-ether anesthesia was also administered. During preparation for section she was maintained only by those resuscitative efforts available in the early 1950s for about an hour. The section was either in the late agonal period or after death, and the fetus was beyond resuscitation. This fetus was coded by the working party as dying with the mother. No autopsy was performed so that the possible role of the motor vehicle accident

remains unclear. Neither marrow nor amniotic content embolization can be ruled out from available data.

Comment: This case shows the complexity added both to pathogenesis and the analytic process by late pregnancy physical trauma. Consider the possibilities if a woman with progressive gestosis becomes medically disabled while driving, leading to a vehicular accident.

Extracranial nongestotic hemorrhage (14

This category includes those hemorrhages arising from pathophysiologic changes specifically related to the reproductive tract but in the absence of gestosis or toxemic manifestations and in the absence of amniotic fluid embolism (Table IV). Three basic lesions occur in this group: (1) uterine atony, (2) tears in the reproductive tract, and (3) placental abnormalities. There was only one case of uterine atony, a 25-year-old, gravida 3, para 0, who delivered at home. She began to bleed heavily, was taken to a hospital, and the placenta was delivered in the ER. Irreversible brain damage from exsanguination had already occurred. She died after a coma lasting 4 days.

Lacerations in the birth canal occur frequently but are not often directly lethal. The extreme form of this is a ruptured uterus. This occurred once after oxytocin without superimposed amniotic fluid embolism. The ruptured uterus in a woman with osteogenesis imperfecta is of interest. The mother was a 30-year-old, gravida 2, para 2, who sustained lacerations of the cervix and vaginal vault in her first pregnancy. The second labor was uneventful. Her third labor was characterized by intense contractions especially in the latter half of the first stage, a pattern observed in both prior deliveries. Mild premature separation of the placenta was present; the infant was liveborn, weighed 2800 gm at 38 weeks, and died of hyaline membrane disease. The infant also showed osteogenesis imperfecta. The mother had religious objections to blood transfusions. Fluorocarbonic acid blood substitutes were tried without success; she died 5 hours postpartum.

The placental lesions are those commonly considered to have grave risk for heavy bleeding ab initio, placenta previa accreta, and abruptio placenta. Two of the abruptions triggered the sequence of disseminated intravascular coagulation. One occurred at 26 weeks gestation in a patient with mild gestosis and Rh sensitization. An exchange transfusion was planned, but she was admitted with backache and bleeding that progressed to abruption and Couvelaire uterus. Supracervical hysterectomy was used to control bleeding, and nearly 13,000 ml of intravenous fluids were infused over a 4-day period leading to pulmonary edema. The final patient with abruption, a 31-year-old, gravida 5, para 4, had a ruptured uterus without specific obstetric antecedents. The lacerations went deeply into the adnexal tissues.

5.5.1Miniabstract 8

A 27-year-old woman, gravida 3, para 2, had both prior deliveries by section. She was in the twenty-fourth week of pregnancy with proven marginal placenta previa and early signs of bleeding. Oxytocin induction was begun and delivery was by low forceps. The 822 gm infant lived less than 2 hours. Hemorrhage after delivery was controlled in part by supracervical hysterectomy. Recognition of a cervical laceration led to subsequent excision of the cervix. The surgical specimens revealed partial placenta accreta. Hypovolemic shock intervened and became irreversible with death 5 hours and 10 minutes after delivery.

Comment: This case is a particularly compelling combination of obstetric problems. Marginal placenta previa beginning to bleed in the twenty-fourth week of gestation portends a poor prognosis for the fetus. One might wonder why, in the face of two prior sections. a hysterotomy was not done to relieve the situation. On the other hand, considering the large number of births represented by this 20 year review, this is the only case directly connected to placenta previa as the principal pathophysiologic factor. This might seem like a salutary state of affairs. A commonly noted frequency of placenta previa, however, is about 1 in 300.37 Thus one of 281 maternal deaths is not a significant difference from the expected range. Some authors have given a higher rate for previa, 1 in 168,34 and 1 in 281 is more likely a real difference compared with that baseline.

5.5.2 Miniabstract 9

A 40-year-old woman, gravida 8, para 2, abortus 2, was in the fortieth week of pregnancy. There had been prior cervical surgery; the record was unclear whether this was a conization or a partial amputation. Labor augmentation consisted of 5 IU oxytocin in 500 ml of normal saline solution given over 1 hour. Delivery was an assisted breech extraction with Piper forceps on the aftercoming head. The placenta was removed manually: a lacerated lower uterine segment and cervix and a ruptured uterus were found. Hysterectomy was performed 3 hours postpartum. Death occurred 5 hours after birth and 2 hours postoperatively. Hypovolemic shock was irreversible despite surgical removal of the lacerated uterus. The 2892 gm infant survived.

Comment: There was no evidence of amniotic fluid embolism despite the close match of the case to the profile of higher risk for amniotic infusion and the excessive use of oxytocin.

The normal variations in anatomic arrangements of human placentation and the normal adaptations of the reproductive tract in labor carry an inherent risk of hemorrhage. This risk would be diminished by avoidance of excessive force in labor management. Abruption other than that in preeclampsia may be an apparently random event with some relationship to higher parity, just as previa seems to be.37 Three of these four

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patients were para 4 or more in the index pregnancy. Atony is also more likely in the multiparous uterus. The relationship of osteogenesis imperfecta to tears in the birth canal remains problematic because only one case occurred, but the repetitive nature of the lesions opens the matter to speculation. It may be that a more general mesenchymal disorder is present in some individuals, such that the soft tissues of the canal tear more readily.

5.6 Obstetric neoplasmas (6 cases)

Neoplasms of the placenta represent the only true obstetric tumors. Theoretically the placental hemangioma, or chorangioma, could lead to maternal death through the mechanism of abruption or retention as a placental polyp. Chorangiomas large enough to be seen with the unaided eye would qualify, but these occur in only about 1 in 1200 pregnancies,44,45 and such sequelae are rare. The working party found no example of this phenomenon in more than 1100 maternal deaths between 1949 and 1985. Neoplasms of the placental site trophoblast would also be an unusual form of obstetric maternal death,18,45 but none were found on review by the committee during this 36-year period. The more expected trophoblastic tumors did occur. There were five cases of choriocarcinoma, all from the first half of the 20-year period specially emphasized in this report, and one example of fatal chorioma, grade 1 (chorionic molar pregnancy). Five cases are insufficient for a profile analysis, but some interesting aspects are remarkable.

Two women were in their first pregnancy, ages 23 and 21 years, respectively. One was diagnosed at 12 weeks as having chorioma, which was evacuated and interpreted as grade 1. Six months later the human chorionic gonadotropin titer was zero, but soon thereafter an explosive metastatic spread of choriocarcinoma was recognized and she died before the end of the year. The second primigravida went to term and delivered a healthy child. Eleven months later she was diagnosed as having metastatic choriocarcinoma and lived less than a month thereafter. The survival for both cases of primigravidous choriocarcinoma patients was less than 1 year.

The third patient was a 27-year-old, gravida 4, para 4. On the third puerperal day hemoptysis occurred, which was attributed to pulmonary tuberculosis. Eventually the correct diagnosis of metastatic choriocarcinoma was established; she died on the one hundred thirty-third puerperal day.

The remaining two patients were of high parity. One was 40 years old, gravida 9, para 8. Eight years earlier curettage removed a grade 2 chorioma; 2 months after diagnosis hysterectomy was done. Approximately 1 year before death intraabdominal hemorrhage occurred and was caused by metastatic liver lesions. Chemotherapy was applied generally and into the hepatic

artery to no avail. The other was a 52-year-old woman, gravida 10, para 9. At age 47 years a grade 1 chorioma was removed by evacuation. Recurrences were at 2 and 3½ years later; the first was treated by hysterectomy and the second by local vaginal excision. At 18 months further metastatic lesions were discovered in the brain. She died soon thereafter.

5.6.1 Miniabstract 10

The patient was a 24-year-old primigravida with an initial prenatal visit in the ninth week of pregnancy. She was of average stature and in apparent good health. Shortly thereafter two episodes of vaginal spotting occurred and in the eleventh week the fundus was palpated at 8 cm suprapubic. Hospitalization for diagnosis and treatment followed. She died suddenly 5 days later. Autopsy revealed a locally invasive chorioma with Clostridium sepsis, disseminated intravascular coagulation, and widespread gas embolization.

Comment: There was no reason from the available clinical information to suspect illicit or unreported instrumentation of the uterus, the case occurred in the early 1970s. Instrumentation is not a necessary part of pathogenesis because local injury to the bowel wall from pelvic spread of invasive chorioma is a sufficient portal of entry for clostridial species normally found in the colonic flora. Neither the clinical studies, for which there was little time, nor the autopsy addressed the question of specific immune deficits or possibilities such as familial neutropenia, which predispose to clostridial infections once outer barriers are breached.

5.7 Therapeutic misadventure (7 cases)

One case was classified here rather than under anesthetic misadventure because the committee was unable to learn what anesthetic agent was used. Direct attribution was, accordingly, not possible. The case in question was that of a 20-year-old primigravida in the fifteenth week admitted for termination of pregnancy by dilation and curettage; cardiac arrest occurred during the preliminary examination under anesthesia (EUA). The woman never regained consciousness.

The other cases in this group included three additional pregnancy terminations, all by saline solution injection into the amniotic sac. Two were very similar. One patient was known to have lupus erythematosus at age 21 years and in the first pregnancy at 19 weeks. She lapsed into stupor 4 hours after receiving 165 ml of 20% saline intraamniotically. The second was 23 years old, of unknown parity, at 22 weeks. The procedure was begun in another state. She was transferred obtunded and died soon after admission. The third death after saline injection was also 23 years old, gravida 3, para 2. This procedure was also begun out of state. On admission partial delivery of the presenting breech was found. The placenta was 75% separated, and the patient died from exsanguination.

The remaining three cases are of interest for the range of possible problems in pregnancy perhaps not

always considered as sources of difficulty. The first was a 36-year-old woman, gravida 4, para 0, with three prior spontaneous abortions. A class 4 smear and extensive Schiller negativity on the cervix prompted admission for conization. Submucous injection of 1 ml of 1:1000 epinephrine with 9 ml of saline (not local anesthetic) was followed by almost immediate apnea. Resuscitation was unsuccessful, and she was declared dead 47 minutes later. Cervical tissue revealed probable early invasion by epidermoid carcinoma. Bolus injection into the hypervascular cervix of pregnancy seems the most likely mechanism of what was an overdose even in the most aggressive treatment plan and very much so with the intended vasoconstrictive effect for local anesthesia.

The second was a possible idiosyncratic reaction to methylergonovine maleate. The patient was 26 years old, gravida 3, para 2. No antecedent obstetric abnormality or positive family history was found. Both prior deliveries were uneventful. Labor and delivery were normal. Eight minutes after birth of the surviving 2860 gm infant, intramuscular methylergonovine, 0.2 mg (the normal dose), was given for third-stage uterine contraction. In a "few minutes" she developed headache, after which her blood pressure rose to 180/110, and 3 hours postpartum a grand mal seizure occurred. She lapsed into a coma and died 4 days and 111/2 hours later. Careful examination of the brain revealed no aneurysms but a 100 ml blood clot in the right lateral ventricle. There were no heart lesions. Neither renal nor hepatic lesions of gestosis were present. As in the case noted directly above, the possibility of direct intravascular injection cannot be excluded.

The final case in this group involves lapse of medication effects. A 19-year-old woman with a strong family history of epilepsy was diagnosed as being epileptic 2 years before her third confinement. Delivery was spontaneous after spinal anesthesia. The first delivery was at age 16 years; the second delivery was at 17 years of age after a diagnosis of gonorrhea during pregnancy. She had been maintained on phenytoin sodium, 300 mg three times daily; immediately postpartum this was reduced to 100 mg three times daily; patient compliance was not documented. She died in status epilepticus on the eighth puerperal day. Postmortem study revealed no phenytoin in the blood. No other likely medication was found either. The autopsy was otherwise unrevealing.

6.0 Pregnancy: A probable major contributory factor 6.1 General

In this major section, the fact of pregnancy is considered to be contributory, whereas the mortal pathogenetic factors are not pregnancy exclusive. This situation will be seen especially in Section 6.3, anesthetic misadventure, and in Section 6.5, pulmonary thromboembolism. Inclusion of puerperal sepsis here is possibly debatable, yet the working party noted an even

larger group of infectious illnesses that did not have the reproductive tract as their portal of entry. Those cases are in the third group (Section 7.0). A case might be made for placement of this group in Section 5.0 were it not that acute bacterial endometritis leading to parametritis sometimes occurs without pregnancy. Moreover, not all puerperal uterine cavities become infected, although the lower birth canal cannot be effectively sterilized for birthing. Thus although pregnancy is a major factor in these cases, it is not a unique one, which was the criterion for the general classification as noted in Section 5.1.

Overall, 54 pregnant or puerperal women died with these findings. This is 19.2% of the series grand total of 281 cases.

6.2 Air embolism, post coital (1 case)

The patient was a 22-year-old primigravida in approximately the twenty-eighth week of gestation. So far as known (and the autopsy did not reveal otherwise) the prenatal course to that time was normal. She collapsed during heterosexual intercourse. She was dead on arrival at a nearby hospital. Autopsy revealed 30% placental separation. There was foamy froth in the right atrium and the right ventricle of the heart, which was otherwise normal. The lungs were wet, weighing 1300 gm together (about three times normal for her age); amniotic fluid embolism was absent. The uterine cavity contained a liter of admixed blood and amniotic fluid. There was no lesion in the brain.

This pathogenetic mechanism, although rare, is not unique.5

6.3 Anesthetic misadventure (13 cases)

These patients are delineated in Table V. The first case might have been included in the group of therapeutic misadventures noted above in Section 5.7 but was assigned here by the working party because of failure of the patient to recover from anesthesia without overt signs of specific intraanesthetic events, such as cardiac arrest during an EUA. In this case, some of the anesthetic and analgesic agents were known.

The principal anesthetic problems during obstetric procedures are those of anesthesia for surgery in general. There were five cases of pulmonary aspiration; two were so massive they caused immediate ventilatory obstruction with eventual death from combinations of pneumonia and cerebral hypoxic injury. Lesser degrees of pulmonary aspiration are followed by pneumonia (from the foreign material inhaled, including gastric secretion), which often produces inexorable destructive inflammation. There were two such patients both surviving 2 weeks. Problems with anesthetic agents such as multiple serial anesthetics, failed spinal anesthesics, and spinal overdoses ("high spinal") make up a good share of these cases (5 of 13), one of which was also marked by difficult intubation and cardiac arrest. Another example of this situation led to subcutaneous em-

Table V. Maternal deaths from anesthetic misadventures

Type or mechanism (puerperal interval)	Anesthesia, medications	Delivery route	No. of cases	
Pregnancy termination, 10 wk (10 hr postabortal)	IV narcotic, Innovar,* di- azepam, unknown final anesthetic		. 1	
Aspiration Massive, obstructing			5	
5 hr	Methoxyflurane + nitrous oxide (nitrous)	Forceps	1	
6 days after hysterectomy first trimester	Nitrous oxide + fentanyl	Surgical	. 1	
Pneumonia				
13 days	Epidural, nitrous oxide + leaking endotracheal tube	Section		
14 days	Nitrous oxide just after a meal, then paracervical	Forceps	1	
Self-extubation, gestosis	<u>-</u>	•		
22 days	Nitrous oxide	Section	1	
Cardiac arrest, difficult intubation			2	
37 hr postpartum, 90 min after tubal ligation	IV thiopental sodium	Vaginal	1	
Postmortem section	2 failed spinals, attempted nitrous oxide + ether	Undelivered	1	
Multiple serial anesthetics (4 hr)	Methoxyflurane + nitrous oxide for delivery, cy- clopropane for hysterec- tomy 2 hr postpartum	Vaginal	1	
Spinal anesthetic overdose Isolated	,	•	3	
10 min	2 spinals, 5 min apart	Forceps	1	
4.5 days	High spinal, respiratory arrest	Section	.1	
Congenital heart disease (2.5 hr)	High spinal, severe hypo- plasia of aorta	Section	1	
Subcutaneous emphysema, gas embolization (2 days after sal- pingectomy for ectopic preg- nancy)	Enflurane + thiopental sodium		1	

IV, Intravenous.

physema during salpingectomy for ectopic pregnancy. The final case was a cardiac arrest during intubation for puerperal tubal ligation.

6.3.1 Miniabstract 11

The patient was a 23-year-old primigravida at term. No evidence of gestosis was present. Onset of labor was spontaneous and no oxytocin was used. Transient fetal bradycardia was seen at 7 cm cervical dilation; artificial rupture of membranes revealed meconium staining of the amniotic fluid. Two distinct spinal anesthetics were administered 5 minutes apart; delivery occurred 15 minutes after the second spinal. The 3430 gm infant did well. The mother died 10 minutes after delivery. Autopsy uncovered neither congenital heart disease nor amniotic fluid embolization. The possibility of sickle cell crisis was not specifically addressed but seems unlikely from the available information. This appears, in retrospect, to be a clear example of medullopontine spinal anesthetic effects from a double dose of agent. The patient had not been intubated for ventilation.

6.3.2 Miniabstract 12

The patient was a 30-year-old primigravida at term. She was obese, weighing 122 kg (273 pounds), and was a pack-a-day

smoker. Borderline gestational diabetes mellitus was present. After 14 hours of oxytocin induction, the cervix was dilated 4 cm with a high presenting part. Two spinal anesthetics were attempted, but neither seemed to provide anesthesia for the contemplated abdominal delivery. Intubation for nitrous oxideether anesthesia was also unsuccessful, and the mother went into cardiorespiratory arrest. Resuscitation failed. Section delivery began a few minutes before the mother was declared dead, but she was in a late agonal state. The infant survived. Autopsy revealed hepatosplenomegaly of uncertain cause, nodular goiter, and acute renotubular necrosis. Some renal tubular regeneration was present, indicating an antepartum lesion or injury not otherwise identified. The possibility of environmental toxic exposure was not considered. The underlying metabolic problem and the renal lesions undoubtedly were burdensome, but the terminal events were set in motion by attempted anesthesia. The case has elements of a complex pathogenesis that will be discussed in Section 7.3 later.

6.4 Congenital cardiovascular disease (7 cases)

This group presents some of the most challenging prospects for future consideration. Six women died in

^{*}Combination of opioid fentanyl citrate and neuroleptic droperidol.

Table VI. Maternal deaths from pulmonary thromboembolism: special added factors

Embolism, not in labor			3
First trimester, 16 days after salpingectomy	1		
Second trimester, 17½ wk, 2 days after ovarian cystectomy	1		
Second trimester, 27 wk, phlegmasia ceruleus dolens, bilateral	1	1	
Embolism, early puerperium, 2½-50 hr post- partum		; •	7
Death at 9½ hr, incidental renal tumor: an- giomyolipoma			
Death at 42½ hr, vein ligation and hysterectomy at 10.5 hr postpartum	1	i	
Death at 50 hr after hysterotomy for pregnancy termination, 22 wk	. 1		
Otherwise unremarkable	4	•	
Embolism, late puerperium, 8-50 days postpartum			6
Death at 4 wk, embolism 8-10 days after de- livery	1		
Otherwise unremarkable	. 5		

the final 7 years of the time-limited study. The sole exception occurred in 1968 with the principal lesion of ventricular septal defect. The patient was a 21-yearold, gravida 1, who was delivered by low forceps in the thirty-ninth week with the use of spinal anesthesia. No surgical repair of the heart had been done, she was known to have pulmonary hypertension, and the hemoglobin was often above 16 gm/dl. The early puerperium was apparently unremarkable when cardiac arrest occurred 85 hours postpartum; she died an hour later.

All but one of the remainder are similar. The unifying point is pulmonary hypertension, sometimes as part of Eisenmenger complex and sometimes with other cardiac anomalies. The women were of low gravidity, 1 or 2, and none of the secundigravidas had living children. They were at or near term and went through labor without difficulty. The postpartum cardiovascular adjustments appear to have imposed an irretrievable burden, and the other five women died at 48 hours and from 3 to 8 days after giving birth. An interesting finding, of possible relevance, is that several of these patients suffered both fever and chills after delivery. No specific localizations of infection were found in any of these cases. Their ages ranged from 20 to 24 years.

The seventh case was somewhat different. This was a 31-year-old woman, gravida 4, para 3, who died rather suddenly 10 weeks after giving birth. She was found to have Barlow's syndrome, mitral valve prolapse, along with patent foramen ovale. Although a population-at-risk statement is not possible from this experience, clearly established pulmonary hypertension will mean a difficult puerperal interval irrespective of its origin. This is significant for preconceptional counseling.

6.5 Pulmonary thromboembolism (16 cases) A time sequence of these cases is provided in Table VI. The three women not in labor all had additional predisposing conditions. Pelvic surgery is a known factor in the development of pulmonary thromboembolism for both men and women. The added changes in some clotting factors caused by pregnancy accentuate this risk. This accounts for two of these cases. One of them was barely into the second trimester at 17.5 weeks. Autopsy indicated preliminary episodes of multiple small pulmonary embolizations.

The third woman not in labor has a different sort of risk. She had severe bilateral saphenous and varicose femoral venous disease. The hematocrit was only 18%, the hemoglobin 5.7 gm/dl, and the blood urea nitrogen was 97 mg/dl. Massive pulmonary thromboembolism occurred in the twenty-seventh week of gestation. No prenatal care had been sought; she spoke no English. Her Iberian origins strongly suggested a concurrent Cooley's anemia in retrospect, but this was not considered during the brief medical contact before death. The elevated blood urea nitrogen was likely due to the occasional pyelonephritis accompanying thalassemic syndromes.

The time frame for puerperal pulmonary thromboembolism is of particular interest. For practical purposes the earlier group, 21/2 to 50 hours postpartum, does not overlap the period of amniotic fluid embolization, although in the latter syndrome women do not always die at the time of the event (Section 5.2). The late group includes some markedly delayed effects, 28 and 50 days were the two longest puerperal intervals. The patient who died 50 days after giving birth had an unusual location for thromboemboli, the left renal vein, with associated early infarction of the left kidney and left adrenal infarction.

These women tend to be in their middle thirties with appropriate high gravidity. Gravida 6, 7, 10, and 12 are represented, with reduced parity, 3, 4, 8, and 7, respectively. To some extent the younger mothers

showed a similar fall off of parity. For example, one woman was age 27 years, gravida 3, para 0; another was 20 years old, gravida 5, para 2.

6.5.1 Miniabstract 13

The patient was a 27-year-old woman, gravida 3, para 0, abortus 1, termination 1, at purported term. She weighed 123 kg (273 pounds) and was 155 cm (61 inches) tall. Her blood pressure ranged about 140 to 150/90 to 100; she had lost 9.8 kg (22 pounds) to this time and showed variable proteinuria. There was meconium stain at time of artificial rupture of the membranes and a section was done after onset of fetal bradycardia. For 5 days she had a low-grade fever without localization. On the sixth day the groin became red. On the eighth day superficial thrombophlebitis occurred, and on the eleventh day chest pain developed. Immediate embolectomy was performed, and a vena caval tent and aortic balloon were inserted without effect. She died shortly after surgery. The infant weighed only 1800 gm and was considered appropriately proportioned for size, suggesting that the pregnancy was short of term. The infant survived. There was a large saddle embolus in the main pulmonary arteries.

Comment: In many ways this case is in a poorly defined middle range sequence, a period after delivery in which no specific disease seems to arise, despite some unusual clinical features, for example, extreme obesity. The low-grade fever may have kept her in the hospital (many patients these days might be at home before the sixth day), but that did not avert a disastrous outcome.

6.5.2 Miniabstract 14

The patient, a 36-year-old, gravida 5, para 4, was a two to three pack-a-day smoker with chronic bronchitis. She weighed 105 kg (235 pounds). The first four pregnancies were uneventful. Vaginal bleeding occurred in the twenty-third week of pregnancy and then again in the twenty-sixth week. She was seen in silent labor and was found to be with twins. Twin A delivered spontaneously after which there was a brief delay; the head of twin B was in the vagina and the body was in the lower uterine segment at the time of massive fatal pulmonary thromboembolism. Twin A survived only 2 hours; the birth weight was 510 gm. Twin B died with the mother. It was not weighed but must have been smaller because it was only 18 cm long from crown to heel. Autopsy revealed lymphocytic thyroiditis in addition to the obesity and bilateral pulmonary thromboembolism.

Comment: One feature in common with the previous case abstract is whole-body obesity. This seems to be a risk factor for thromboembolic events. The volume capacity of the deep veins of the lower body and legs is enhanced by obesity; relative inactivity occasioned by body bulk may also be important here.

There was one patient who died of pulmonary thromboembolism in whom autopsy revealed a large renal hamartoma, or angiomylipoma. This was as large as a kidney itself. There was no evidence of thromboembolism arising from the tumor. The patient had had a splenectomy many years ago for congenital hemolytic anemia. This tumor has been implicated in maternal death through spontaneous rupture.¹⁷

6.6 Renal failure (2 cases)

Both patients had end-stage nephrosclerosis. One patient was a 39-year-old, gravida 8, para 4, abortus 2. She was admitted in the fortieth week with 50 mg/dl proteinuria. Progressive labor led to spontaneous delivery of a surviving infant using spinal anesthesia. A first-degree vaginal laceration was present. The past history suggested a diagnosis of "nephritis" 6 years earlier. Preterminal blood urea nitrogen was 140 mg/dl; creatinine was 7.5 mg/dl. Neither peritoneal lavage nor hemodialysis had any beneficial effect. Marked facial edema occurred agonally. She died on the twenty-seventh puerperal day. Autopsy revealed progressive nephrosclerosis, marked fibrinoid arteriosclerosis, and many cortical and medullary intrarenal casts. Both kidneys were small, weigning 145 gm together. The lungs were massively wet, weighing 1950 gm jointly. Microscopically a typical uremic pneumonia was present.

The other patient was a 28-year-old, gravida 7, para 6, also in the fortieth week of pregnancy. Urine albumin had been as high as 900 mg/24 hours. She was hypertensive. She had a previous term stillborn. Her weight went up an unknown amount to 85 kg (190 pounds). At full cervical dilation a spinal anesthetic using mixed pontocaine and xylocaine was instituted; the membranes were ruptured shortly thereafter revealing a large volume of green fluid. Ten to 20 seconds later she became apneic; cardiac arrest followed. The 3544 gm infant was immediately delivered by outlet forceps and did well, with an Apgar score of 6. The mother died 55 minutes after delivery. Autopsy showed latestage mixed renal disease with pyelonephritis, arteriolar nephrosclerosis, and large vessel arteriosclerosis. Careful search of the lungs failed to demonstrate any amniotic fluid embolism (AFE) or meconium deportation.

Despite matching the profile of AFE, this second patient seems to have had another pathophysiologic disturbance. In retrospect, the possibility of high spinal anesthesia seems a useful alternate mechanism, but no unusual sensorium was commented on in the medical record. This might have been obscured by onset of the agonal state. The renal lesions, on review, seem as severe as those in many patients dying of primary renal failure, albeit this death is not one readily so attributed except by absence of some other more compelling cause. With only two cases at hand, no profile of renal failure in late pregnancy is possible. The first case is one of obviously progressive renal failure; the second is perhaps best considered an example of combined abnormalities in which underlying renal disease added to the stresses and challenges of labor, anesthesia, and delivery.

6.7 Obstetric infection with sepsis (16 cases)
This particular category is related to the cases dis-

Table VII. Maternal deaths from infection/sepsis arising from pregnancy: Puerperal endometritis with extension and/or sepsis

Localization (puerperal interval)	Principal organism (s)	Delivery route	No. of cases
Placenta accreta (2.8 hrs)	Aerobacter	Vaginal	1 (1)
Postabortal		8	. ,
(9 hr)	Clostridium	Surgical (*)	1
(47 days)	Clostridium, Bacteroides	Surgical (*)	1
Postintrauterine contraceptive device		0 ()	(2)
Peritonitis and sepsis (50 hr)	Streptococcus	Hysterectomy at 13 wk	1
Septic myocardial infarction (16 days)	Escherichia coli	Vaginal at 23 wk	1
Puerperal sepsis (5 hr) Placenta caught in vagina, first admission 3 days earlier			(10)
Bacteroides, Staphylo	ococcus	Vaginal	1
(11 hr) Amniochorionitis	Streptococcus	Vaginal	1
(5 days) Peritonitis	E. coli	Section	1
(5 days) Febrile seizures	Streptococcus	Vaginal	1
(10 days) Peritonitis	Colonic flora	Section	1
(14 days) Persistent sepsis	Streptococcus, Staphylococcus	Vaginal	1
(14 days) Pulmonary hemorrhage	Streptococcus	Forceps	1
(14 days) Breast abscess	? Viral syndrome	Vaginal	1
(16 days) Subdiaphragmatic abscess	Bacteroides, Streptococcus	Forceps	I
(23 days) Persistent sepsis	Proteus	Vaginal	1
Rectovaginal septal hematoma	Colonic flora	Vaginal	I (1)

^{*}Dilation and curettage.

cussed later in Section 7.7 and represents 30.8% of all cases ascribed to infection or sepsis as the principal pathogenetic mechanism. Placement in this group is based on infections arising directly in the reproductive tissues affected by pregnancy. Table VII lists a number of attributes of the 16 cases: tissue localization, time intervals, principal organisms, and route of delivery. The table makes clear that even in this era of intense antimicrobial therapy puerperal sepsis of the classical type still occurs. The proportion between section and vaginal delivery cannot be further examined as to significance in the absence of definitive data on the frequency of section births for the period. The Commonwealth of Massachusetts did not begin to maintain birth statistics of sufficient detail for general research use until 1969.8 More importantly, data records respecting section deliveries began in 1982. In 1982 14,677 infants were delivered by section of a total 75,749 live births, or 19.38%, a value so close to the 2 of 10 as to be indistinguishable. The rate for 1983 was 20.05%; for 1984 it was 21.03%; for 1985 it was up to 22.01%. Because the time interval chosen for study by the working party for this report considerably antedates this time period, these rates may exaggerate the situation. A sharper rise in use of sections was noted in the late 1970s so that a linear model does not pertain. The maternal death factor suggests no special predilection for severe puerperal sepsis with the use of section delivery, even though the accumulative death rate is somewhat higher than the aliquot of deliveries by section over this 20-year period.

Other infectious cases include two of the earliest recorded maternal deaths from intrauterine contraceptive devices in place, which led to regulatory action eventually removing them from the market. Placenta accreta represents a special form of acute puerperal endometritis but has been separated in the table for emphasis. The lesions of puerperal sepsis at autopsy are fairly characteristic. These are principally infected thrombi in the parametrial veins. In some cases a continuous thrombotic cord is seen in the ovarian veins extending right into the inferior vena cava. The placental site will show abundant thrombosis, active and subacute inflammation, and very often overt bacterial colonies. These are on the uterine mucosal surface, which is an unhealed placental site often beyond the reach of active circulation and thereby unaffected by antibiotics.

6.7.1 Miniabstract 15

This patient was a 23-year-old woman, gravida 4, para 3, delivered in the thirty-sixth week by outlet forceps under spinal anesthesia. One of the three prior pregnancies went to 36 weeks; the others were term. The prenatal course was fully normal except for the onset of labor, which produced a living infant weighing 2722 gm. She was discharged routinely. All the older children had chickenpox at the time of discharge, but the mother's own history on this was uncertain. She was readmitted 14 days after delivery with pain in the right leg, which had lasted for 3 days. Two days before readmission she had complained of hemoptysis. Right leg thrombosis was diagnosed; she was recognized to be in a highly toxic and septic state, had disseminated intravascular coagulation, and died soon thereafter of massive pulmonary hemorrhage. Both uterine and blood cultures were strongly positive for β -hemolytic streptococci. No evidence for varicella pneumonia was adduced at autopsy.

Comment: Some evidence exists to suggest that β-hemolytic streptococcal sepsis is on the increase or has become more common puerperally, as reflected in a clear rise in neonatal streptococcal sepsis. ¹⁶ Injured capillary endothelium in sepsis caused by this organism makes hemorrhage an extension of the profile of septic lesions, and the lung is a prime target. Although no evidence was found to implicate varicella, mixed or synergistic viral-bacterial infections are also suspect and should be considered in such overwhelming cases.

6.7.2 Miniabstract 16

A 22-year-old primigravida delivered spontaneously in the forty-third week of pregnancy under light inhalant anesthesia. The infant weighed 3572 gm and did well. The prenatal course was marked by spontaneous pneumothorax of unknown cause in the first trimester. Vaginal bleeding 1 hour postpartum led to discovery of a lengthy vaginal laceration and a hematoma forming in the rectovaginal septum. This grew rather large and the patient went into shock. Hypogastric artery ligation was used followed by hysterectomy and bilateral adnexectomy. The postoperative course was stormy; the patient went into shock and sustained renal tubular necrosis, hemiplegia, and ureteric obstruction requiring renal exploration. She died 42 days postpartum after sepsis from mixed colonic flora. Autopsy revealed an exposed ligature in the rectal mucosa around a major rectal artery and demonstrated severe confluent bronchopneumonia with abscesses. There was marked hepatomegaly with zonal necrosis. The exposed rectal ligature is an obvious chronic portal of entry for the mixed flora.

Comment: If ever there was an argument for careful surgical technique including close postoperative surveillance, this case is surely support. It also denotes the risk of laceration of the vagina without obstetric instrumentation. It is a reminder that posterior vaginal injury is tantamount to anterior rectal injury. The case also illustrates the sequences of persistent sepsis with the hazard of pulmonary localization of suppurative organisms. The presence of much blood in the rectovaginal septum also compounded the clinical problem because the breakdown of retained blood aids both aerobic and anaerobic organisms to become established.

7.0 Intercurrent disease or pregnancy an uncertain or minor factor

7.1 General

Slightly more than half of all maternal deaths for the time period 1966 to 1985 fall into this group (144 cases of 51.2%). As the general listing in Section 4.4 makes clear, this is an extremely diverse set of medical, surgical, and obstetric factors. Because the listing in Sec-

tion 4.4 is complete with respect both to categories and principal individual factors, additional tabulation will not be undertaken. The working party divided this large number of cases into 12 subgroups, a few of which are limited in both size and scope. The dominant subgroups, with their section designators, are accidental deaths, acquired heart disease, complex pathogenesis, nongestotic intracranial vascular incident, non-obstetric neoplasm, and infection and sepsis. At least two cases from each of these six subgroups will be abstracted.

A more precise attribution may be possible in the future when more is known about the influence on various organs and tissues of the pregnant state when intercurrent disease manifests. Several specific examples of this potential will be developed in each section pertinent to that subject.

7.2 Accidental deaths (24 cases)

The clearest pregnancy-related accidental death occurred when a 25-year-old woman in labor fell down a stairwell in a hospital. She had been lightly sedated initially, and during an interval when labor lessened, she apparently wandered onto the landing. She had multiple lacerations of the head, contusions, and a presumed concussion. She went rapidly into a fatal coma and was delivered of a 39-week living infant by postmortem section. The infant died a day later. Toxicologic studies showed blood levels of meperidine and secobarbital well below the therapeutic ranges.

The other accidents bear variable time relationships to pregnancy. In general the committee did not go beyond the stated facts of each case as they became known at the time. The working party engaged in limited psychologic autopsy interpretations. The first miniabstract for this section embodies this aspect.

7.2.1 Miniabstract 17

The patient was a 22-year-old, gravida 3, para 2, woman who had an uneventful obstetric prenatal history, labor, and delivery that was spontaneous using cyclopropane anesthesia. She was separated from her husband, possibly since before this pregnancy. The child was healthy and survived the incident that follows. Forty-four days after giving birth she was visiting a neighbor's home. Present were the host and his wife, this woman, and two other adult guests. At approximately 9 PM the host apparently left the living room. When he returned, he shot her, his wife, and both other guests, and then shot himself. The psychodynamic implications of this disaster offer several possibilities for follow-up, which was not done because the obvious perpetrator was himself a victim. A blood group profile of at least the woman, the host, and the child might have shown what would likely be concluded to be a selfish or psychotic motive on his part.

Comment: Several other homicides offer similar possible understanding. For example, why would someone seek to commit murder by hire 9 weeks postpartum?

Even more tragic in a way, a mute woman with congenital deafness reported "spousal" abuse by her livein male friend. These reports were not given credence by the local authorities until after she was found murdered by multiple stab wounds to the chest at about 20 weeks' gestation during her second pregnancy. The male friend was charged.

7.2.2 Miniabstract 18

The patient was a 19-year-old primigravida in the thirty-seventh week of pregnancy. She was one of several persons injured in a collision between a bus and a car that she was driving at 2 AM. She was admitted to the hospital at 3:20 AM; immediate laparotomy revealed rupture of both spleen and uterus, a lacerated liver, and the fetus and placenta lying free in the abdomen. Supracervical hysterectomy was done in extremis. The mother died several hours later from the effects of massive intraperitoneal hemorrhage.

Comment: Impact trauma from vehicular collisions may differ in specific ways because of displacement of organs during pregnancy. This case involved a steering wheel injury across the upper abdominal rim. The diagnosis and treatment of mass internal injury in pregnancy must contend with both the organ displacement phenomenon and altered vascularity. Fetal salvage was precluded by uterine rupture and abruption.

7.3Acquired or nonanomalous heart disease (13 cases)

Arteriosclerotic heart disease has major implications in pregnancy. We have already mentioned one patient with gestosis also showing familial hypercholesterolemia, severe coronary artery disease, and myocardial infarction. This group contains five other cases without the background of gestosis. This makes arteriosclerotic cardiovascular disease more common as a cause of maternal death than so-called gestational myocardiopathy of which only two cases were found during this 20-year period. The generally decreasing significance of rheumatic heart disease is reflected in only two related cases despite the higher frequency of the condition in the northern climate of Massachusetts. Single examples each of unusual forms of cardiovascular disease were found. One had Marfan's syndrome and aortic dissection, and one had kyphoscoliotic heart disease. The latter woman died 4 days after attempted intrauterine fetal exchange transfusion and 59 hours after section delivery of a stillborn fetus. There was one example of coronary artery dissection. This woman was a 35-yearold primigravida with a history of migratory polyarthritis and a diagnosis of rheumatic fever at age 5 years. She was delivered by section and received no penicillin after surgery. She died suddenly 62 days after giving birth. Autopsy reveals massive left ventricular myocardial infarction and congestive failure caused by a dissecting aneurysm of the left coronary artery. The senior author has seen this lesion twice recently in women of very similar ages, one a 36-year-old multigravida 7 years past her most recent pregnancy20 and the other also a 36-year-old woman of uncertain parity 1 week postpartum.

7.3.1 Miniabstract 19

The patient was a 21-year-old primigravida who had premature labor in the thirty-fourth week of gestation. Delivery was by low forceps under spinal anesthesia. Urinary tract infection manifested postpartum. She signed herself out against medical advice. Twelve cays postpartum she was seen again and had an apical systolic murmur. Two days later she had cardiac arrest but was resuscitated. A retrospective history revealed migratory joint pains and pharyngitis suggestive of a rheumatic component. A working diagnosis of acute mitral insufficiency was made, and a Starr-Edwards mitral prosthesis was used to replace the mitral valve. At surgery septic rupture of a mitral papillary muscle was found. She died on the nineteenth puerperal day, 4 days after surgery.

Comment: Urinary tract infection is a somewhat unusual, but not an unlikely, complication. It is probable that premature labor was due to the same factor of urinary tract infection. The placenta was apparently not examined for acute inflammatory disease.

7.3.2Miniabstract 20

A 35-year-old primigravida has "known" rheumatic heart disease since 5 years of age. Major episodes of migratory polyarthritis occurred at ages 21 and 27. She weighed 65 kg (145 pounds) and was 157.5 cm (62 inches) tall. She was delivered in the forty-first week by section using spinal anesthesia. A 3250 gm infant survived. No penicillin was used in the puerperium. She was readmitted in extremis, placed in intensive care, and died on the sixty-second postpartum day. Autopsy showed a dissecting aneurysm of the left anterior descending coronary artery, massive global infarction of the left ventricle, profound congestive heart failure, and acute renotubular necrosis. No evidence of rheumatic disease was found at autopsy.

Comment: The childhood diagnosis of rheumatic disease often seems suspect to pathologists with experience at autopsies on early middle life adults. Rheumatic and rheumatoid disease can take unusual forms on occasion, and dissecting aneurysm or hematoma of the coronary artery could very well destroy the primary rheumatic focus. Generally, extensive myocardial infarction more than 4 days postictal would make identification of Aschoff bodies difficult. This possibility was not addressed in the autopsy study in this particular case.

7.4 Complex pathogenesis (17 cases)

When pathologists reconstruct the probable sequence of events leading to death, they often find difficulty in weighing the complex factors. If a time sequence is well documented clinically or by prior biopsy or laboratory information, the task is simplified. In the opinion of the working party, one is often limited because of a lack of published prior experience in some particular combination of disorders or events. This is why we have attempted to display a range of lesion potential when the data so permit. Mining this particular set of cases is obviously a prolonged process, and we have chosen several challenging abstracts to illustrate this point. In some instances the complex matrix contains factors discussed elsewhere in greater detail. Classification at this point reflects the belief of the working party that the combination is the key factor and not the individual elements. This group also contains those diseases for which cause and pathogenesis remain unclear

7.4.1 Miniabstract 21

A 26-year-old woman of uncertain gravidity was diagnosed as having lupoid hepatitis^{22, 39} when she was 17 years of age. She carried chronically elevated serum globulins. In the estimated twelfth week of pregnancy she was admitted in extremis with signs of intraabdominal disease, possibly a ruptured viscus or mass hemorrhage. Shock gave way to coma. Autopsy revealed a ruptured saccular aneurysm of the splenic artery, chronic active lupoid hepatitis and postnecrotic cirrhosis, and about 900 ml of intraperitoneal blood. The fetus measured 5.0 cm crown to rump, within the range expected for 11 to 12 weeks' menstrual age.

Comment: Lupus erythematosus remains an enigma with varied manifestations. Because arteritis is a major component of the systemic disorder, it is entirely possible that this particular ruptured aneurysm and hemorrhage arose within the immunologic matrix of lupus erythematosus. The volume, 900 ml, is not enough in its own right to cause hypovolemic anemia leading to shock and death. This outcome must have been the result in part of the effects on the liver of the hepatitis and the ability of the body to respond to and modulate stressor amines, marshal the stores of glycogen, and expand the blood volume. This case is mostly questions; classification under complex pathogenesis seems warranted.

7.4.2 Miniabstract 22

A 31-year-old woman, gravida 3, para 1, abortus 1, was bearing twins in the twenty-ninth week of gestation. She was dyspneic and had signs of right heart failure. A section delivery was performed using an unspecified general anesthetic, and both twins survived. The mother did poorly after delivery and died 25 days later. Autopsy showed puerperal myocardiopathy, scleroderma lung, and chronic visceral congestion.

Comment: Although the survival period postpartum is greater, this case resembles those in Section 6.4 in which pulmonary hypertension and congenital heart disease combined to yield a poor prognosis. This is likely a dual effect of scleroderma, with myocardial fibrosis and interstitial pulmonary sclerosis. In cases of congenital heart disease the long-standing left-to-right vascular shunting produces an arterial component perhaps more immediately productive of right heart failure in the postpartum period. Here the obstructive vascular element is more peripheral and more diffuse.

7.4.3 Miniabstract 23

The patient was a 29-year-old woman, gravida 4, para 3, whose first three pregnancies all went to term; with infants weighing 2835 to 2892 gm (a remarkably narrow range of birth weights). In this fourth pregnancy she developed varicosities, 3+ pitting edema of the legs, 4+ proteinuria, and in the twenty-fifth week she became dyspneic. Iron depletion was noted; marrow erythroid hyperplasia was noted, and the blood pressure rose to 220/130. The diagnosis of lupus nephritis was made and steroid therapy was begun. Three weeks later she was delivered of a 680 gm stillborn infant. She did poorly, with progression of renal failure. Death occurred 36 days postpartum. The autopsy confirmed the basic diagnosis of lupus nephritis. Marked adrenal atrophy as a result of steroid use was found.

Comment: This is a more typical expression of systemic lupus erythematosus, renal failure. Lupus affects the placenta, and a lupus factor may cross the placenta into the fetus.^{23, 35} These matters were not examined in this case.

7.4.4 Miniabstract 24

An 18-year-old primigravida had signs and symptoms of acute appendicitis; the preoperative white blood cell count rose to 15,900/mm3, and an appendectomy was performed in the thirtieth week of pregnancy using a modified abdominal approach and spinal anesthesia. No aspiration of vomitus occurred at or after surgery. Pulmonary edema developed after surgery, and uterine contractions were treated with ritodrine hydrochloride. A questionable pulmonary embolus with pleuritic pain was noted. Two days after surgery the patient died suddenly and undelivered. Autopsy examination indicated fetal death 4 to 5 hours earlier. The surgical specimen showed only focal appendicitis. At autopsy there was focal acute peritonitis surrounding the duodenum and pancreas and segmental adult respiratory distress syndrome in the lung. The possibility of viral pneumonia could not be fully excluded because the case occurred at the end of a major influenzal season.

Comment: In retrospect the ritodrine was probably unnecessary because only Braxton-Hicks contractions were present. Adult respiratory distress syndrome produces lung lesions very similar to influenzal pneumonia before bacteria are superimposed.

Such examples of complex pathogenesis could be extended. The pecularity of some cases is also illustrated by the observation that two cases of sarcoidosis involving the heart occurred, and both cases occurred in the same year out of 20 cases.

7.5 Nongestotic intracranial vascular incidence (26 cases)

Intracranial vascular lesions such as medial defect aneurysms are generally considered more common in women,^{24, 52} whereas arteriovenous malformations are slightly more common in men in general²⁴ while marginally more common in women in the third and fourth decades of life.²⁴ Despite the much lower frequency of clinical manifestations of medial defect aneurysms in women,^{24, 25} the working party, based on prior experi-

ence, came to consider that serious complications derived from these lesions were accentuated by pregnancy. This group is placed here rather than in our second main category (Section 6.0), because of the relative lack of basic tissue studies on the effects of pregnancy on the intracranial arterial system. However, 16 proven or very strongly indicated medial defect aneurysms (those indicated are by x-ray studies absent autopsy confirmation) and five arteriovenous malformations appear to us to be an astonishingly large number in 281 maternal deaths, given the age range and the expected natural history of these lesions.24, 25, 52 The combination is 7.5% of the whole. The known cardiovascular adaptations of pregnancy, in terms of volume and pressure,14 are deemed to augment changes in the vascular wall with net weakening, leaks, and rupture. Likewise, changes in coagulation factors²¹ combined with swings in blood volume or viscosity would predispose either to sinus or arterial thrombosis, accounting for four more patients in this subgroup.

7.5.1 Miniabstract 25

A 28-year-old primigravida with a known patent foramen ovale, who weighed 76.8 kg (172 pounds) and essentially had an unremarkable prenatal course until the thirty-seventh week of gestation, went into labor. Twins were delivered spontaneously with local anesthesia. Presumptive pulmonary thromboembolism occurred about 5 hours postpartum. Heparin therapy was instituted, but central nervous system signs developed, and the patient died on the thirteenth puerperal day. Autopsy revealed hemorrhage in the right cerebral hemisphere and thrombosis of the pelvic and ovarian veins. Widely patent foramen ovale was confirmed. The blood clot in the right hemisphere was a large, irregular $7 \times 7 \times 8$ cm mass. No evidence of arteriovenous malformation was found. An embolic occlusion of the right anterior cerebral artery was found. A diagnosis of paradoxic embolization was made.

Comment: Paradoxic embolization through a foramen ovale is rare. This case is considered to qualify in this section because primary thrombosis was limited to the pelvic region, and the blood clot in the right anterior cerebral artery was clearly an embolus. Interestingly, no embolization of the lung was found.

7.5.2 Miniabstract 26

The patient was a 48-year-old woman, gravida 8, para 4, abortus 3. The most recent delivery was at age 38 years and at 38 weeks' gestational age. The four infants weighed from 3175 to 3629 gm; the infant of the index pregnancy weighed 3884 gm. Birth control hormones were used for a 4-year period ending 3 years before this pregnancy (ages 41 to 45 years); during the interval she had no menses. Five years before confinement the diagnosis of essential hypertension, blood pressure surging to 220/110, was made with a treatment regimen including aldomet and thiazides. These were discontinued after pregnancy was diagnosed. During pregnancy, blood pressure was about 145/90. Labor was managed by amniotomy and oxytocin augmentation. The blood pressure went to 158/110 and later to 178/110. Delivery in the fortieth week was spontaneous using local anesthesia. She was discharged on the sixth puerperal day but collapsed shortly after arriving home. On readmission signs of intracranial bleeding were noted, with progressively increased intracranial pressure. Death occurred 12 hours after readmission. Autopsy showed massive subarachnoid hemorrhage arising from an aneurysm of one posterior cerebral artery, and a large cerebellar hemorrhage. The heart weighed an increased 410 gm and showed biventricular hypertrophy typical of chronic hypertensive disease.

Comment: The association between pregnancy and various intracranial vascular changes needs further exploration.

7.6 Nonobstetric neoplasms (16 cases)

The general subject of malignancy during pregnancy has been reviewed by McGowan²⁷ and others.^{35, 53} Of the list of tumors in this experience, two suggest some more particular relationship to pregnancy, pheochromocytoma and astrocytoma. The former would bear clinical recognition about a possible association because of the role of hypertensive disease in pregnancy.

7.6.1 Miniabstract 27

A 36-year-old woman, gravida 3, para 0, abortus 2, had an apparent diagnosis of gestosis, probably superimposed on borderline essential hypertension. There was trace proteinuria. The blood pressure in early pregnancy went up to 180/110. At 30 weeks' gestation the pressure reached 220/110. A significant psychologic overlay was present in the opinion of a consultant psychiatrist with special reference to blood pressure "cuff anxiety," which delayed hospitalization. Signs attributed to hypertensive encephalopathy developed. The blood level of epinephrine was measured at 51,000 pg/ml (normal, 20 to 97 pg/ml) and norepinephrine at >42,000 pg/ml (normal, 120 to 310 pg/ml) shortly before she died, undelivered, from cardiopulmonary arrest. Autopsy revealed a large left adrenal pheochromocytoma weighing 62 gm; a similar, but smaller, tumor on the right measured 1.2 cm in greatest diameter. The final admission lasted only 71/2 hours, with fetal death recorded 5 hours before the patient died.

Comment: The other example of pheochromocytoma had similar features of clinical course, including an apparent lack of appropriate credence by some health care professionals about the seriousness of the problem.

7.6.2 Miniabstract 28

A 30-year-old woman, gravida 2, para 1, with an uneventful first pregnancy, had weakness of the left side of the mouth, dizziness, and headache. The early signs were present at 24 weeks' gestation and progressed to headache by 34 weeks' gestation. Three days before delivery in the thirty-fifth week of gestation, Bell's palsy developed and the patient became restless. Hemiparesis then occurred. Section delivery was done essentially at the same time as craniotomy. This revealed a large right temporal lobe mass histologically astrocytoma, grade 3. Death occurred 48 hours postpartum. The 2722 gm infant survived.

Comment: The other three cases of astrocytoma had

a number of features in common with this one. One patient was known to have temporal lobe epilepsy and was admitted with "personality changes." She was a 31-year-old woman, gravida 1, in the thirty-second week of pregnancy. She was obtunded on admission and craniotomy and delivery occurred while she remained comatose. The fetus was stillborn, weighing 2155 gm. There was a large right temporal lobe astrocytoma, grade 4, with extensions into both the right and left lateral ventricles. The other two cases were at comparable stages of pregnancy, decompensated in similar ways, and had similar, almost immediate, outcomes. Indeed, the profile of this process is almost so narrow as to serve as a diagnostic matrix if one did not have much in the way of confirmatory tissue evidence.

Of the remaining malignant processes observed in this series, the two patients with mammary carcinoma deserve brief mention. One patient was 44 years old, and the other was 30 years old. Both pregnancies concluded prematurely, one by section and the other spontaneously after an intense course of radiation. Interestingly, the fetus delivered by section died neonatally, and the other survived.

7.7 Infection or sepsis (36 cases)

The patients discussed here relate to those described in Section 6.7 and represent the larger share (69.2%) of all infectious or septic processes in the overall study. Within this group, the largest subgroup is pneumonia, with and without other localizations of the infectious process. This accounted for 10 of the 36 patients. Six specified forms of viral disease were found: hepatitis (four), varicella myocarditis (one), and herpes encephalitis (one). The other major subgroup was endotoxemia from coliform organisms, with related attributes such as intestinal obstruction and peritonitis.

7.7.1 Miniabstract 29

The patient was a 28-year-old woman, gravida 2, para 1. The first pregnancy was uneventful as was the prenatal course in the second until approximately 25 weeks' gestation. At that time the patient noted multiple red skin spots similar to flea bites. Four days later she fell without apparent reason; 3 days later she complained of headache and weakness. She was hospitalized 2 days before delivery after a seizure. Labor began; a 1000 gm infant was delivered spontaneously but lived only 3 hours. The mother died 9 days after delivery. Autopsy showed herpes simplex encephalitis, proved by viral culture, and necrosis of both temporal lobes.

Comment: Herpes simplex infection is an intercurrent infection for pregnancy not often manifested in this fashion, raising the unanswered question about this patient's individual immune competence. The mechanism of onset of labor in the face of evolving encephalitis and other infectious processes remains obscure. Fetal or neonatal outcome will be based on the mixture of gestational immaturity and the depth of maternal sepsis, shock, or nutritional stress.

7.7.6 Miniabstract 30

The patient was a 23-year-old primigravida who had an uneventful prenatal course except for cystitis with positive urine culture (Escherichia coli) at colony count >100,000. She went into labor in the forty-third week of gestation and sustained a deep third-degree laceration with complete tear of the anal sphincter. This was repaired, but perineal sepsis developed. After a stormy course, she was discharged home but came back the next day with signs of pulmonary thromboembolism; some emboli might have been septic. The inferior vena cava and ovarian veins were ligated 16 days postpartum. Pericardial effusion and adhesions then developed. During attempted pericardiolysis the intrapericardial segment of the inferior vena cava ruptured, and the patient died 24 days postpartum. Autopsy revealed septic phlebitis at the site of vena caval tear and organizing serosanguineous pericarditis. The 2948 gm infant survived.

Comment: This case is included to show the range of possibilities in bacterial sepsis in the parturient woman. The infant was not large, and most third-degree lacerations will heal, albeit sometimes slowly. It is evident that a pathogenetic mechanism akin to ordinary puerperal sepsis was at work here in the pelvic veins, but seemingly from a different portal of entry unlike the usual endometrial implantation site. Nevertheless, this case could well be classified in group 2 rather than here, were it not for the highly unusual terminal events and the longer postpartum interval without obvious endometrial signs.

7.7.3 Miniabstract 31

A 30-year-old woman, gravida 5, para 4, had three successive births at 37 weeks' gestation with birth weights of 2722 to 2948 gm; the fourth was at 40 weeks' gestation, also at 2948 gm, but this last child died of neonatal respiratory distress. The mother was diagnosed as having chronic ulcerative colitis, which worsened greatly in her fifth pregnancy, especially beginning in the twenty-seventh week with evidence of multiple colonic perforations and peritonitis. She continued to be febrile and went into labor in the twenty-eighth week; total colectomy was performed 2 days after delivery. Nine centimeters of terminal ileum were removed along with the whole colon. A stormy course led to death 9 days postpartum and 7 days after surgery. Autopsy revealed gastric stress ulcers, residual peritonitis, and bilateral acute bronchopneumonia. The 971 gm infant died after several hours of evident respiratory distress.

Comment: The relatively large inoculum into the peritoneal cavity from multiple colonic perforations poses a special dilemma in pregnancy because of the usual mild suppression of immune mechanisms. Total colectomy is a formidable undertaking as well but has less intrinsic negative nutritional potential for a continuing pregnancy than does extensive surgical bypass of the small intestine.⁵¹ Pregnancy is a complicating factor in this case. The working party accorded it a lesser role, one of aggravation rather than causation, because the disorder appeared during an interpregnancy interval.

7.7.4 Miniabstract 32

The patient was a 38-year-old woman, gravida 7, para 6. Little was known about her previous pregnancies, and she received no prenatal care for the seventh pregnancy. She weighed 111.6 kg (250 pounds) and was 162.6 cm (64 inches) tall. On admission she was noted to be anemic (hemoglobin, 8.9 gm/dl) and hypertensive (150/90). Delivery was spontaneous without anesthesia, and the 3714 gm infant survived. She was discharged home in apparent good condition, but on the sixteenth puerperal day, a cough and fever developed and were treated with antibiotics. Three days later she collapsed, was readmitted to the hospital, and died 5 hours later. Autopsy revealed advanced bilateral influenzal pneumonia (right lung weight, 1050 gm; left lung, 975 gm) and hypertensive cardiovascular disease (heart weight, 550 gm, with left ventricular hypertrophy).

Comment: This death occurred during the usual late winter epidemic season in an especially severe year for influenza.

7.8 Sicklemic-thalassemic manifestations (3 cases) These are uncommon causes of maternal death. Two of the three cases were conjoint sickle cell-beta-thalassemia, but which type beta-thalassemic hemoglobin is unknown. One was a 25-year-old secundigravida who took an overdose of propoxyphene for pain. She had chronic symmetric arthritis at age 5 years and back and retrosternal pain. Chronic anemia was present. Delivery was by section in the thirty-third week; the 2270 gm infant lived only 36 hours. The mother died 30 days postpartum in medullary bone sickle cell crisis. Small marrow emboli were found in the lung and postmortem x-ray films revealed aseptic hip necroses.

The other sicklemic-β-thalassemic case was further complicated by deficiency of glucose-6-phosphate dehydrogenase, thromboses, infection, femoral head necroses; the patient lived 27 days postpartum after delivering a surviving infant at 40½ weeks' gestation.

The third example with sicklemia was also complex. The patient had a history of rheumatic disease and multiple manifestations of the underlying hemoglobinopathy. Seizures denoted the cerebral thrombosis, which was found at autopsy. Death occurred late in the second trimester, with the patient undelivered.

7.9 Status asthmaticus (2 cases)

Status asthmaticus is an uncommon cause of death generally and unusual in this series of maternal deaths. One fatality occurred 77 days after the patient gave birth. The patient was 35 years old and had six essentially normal earlier pregnancies. During the final pregnancy, her asthma was relived. Death occurred in mid-November. The other patient was a 25-year-old woman, gravida 8, para 4, termination 3, with known heroin and alcohol abuse, past asthma, and pelvic inflammatory disease. She was admitted at 311/2 weeks' gestation with a severe asthmatic attack, was treated, and was released. She was rushed to the hospital a week later with another severe attack but was dead on arrival. Postmortem section was done immediately but produced a stillborn fetus. Blood toxicologic studies revealed only a therapeutic level of quinine. Because no quinine had been prescribed, it is likely the patient obtained it as part of a substitute street drug.

7.10 Thrombotic thrombocytopenic purpura (4 cases)

These cases share various aspects of a clinical profile, but not all features were found in all cases. Three of four patients were primigravida; three of four patients were late in the second trimester and did not die in labor; and three of four died with specific hemorrhagic complications. The fourth patient was more complex in each aspect. One patient gave birth in the thirtysecond week of gestation and died on the twelfth puerperal day. One patient was gravida 7, para 6, and carried twins; death was due to ruptured subcapsular hematoma of the liver. One patient had severe chronic renal disease with strong features of lupus nephritis.

7.11 Thyrotoxicosis (1 case)

This 19-year-old woman was probably a primigravida with a known past history of thyrotoxic attacks. She was taking propylthiouracil. Jaundice was present for 10 to 14 days. She was admitted in the twelfth week of pregnancy with fulminant hepatic failure. Autopsy revealed a 100 gm thyroid with diffuse hyperplasia. There was hepatocellular necrosis and regeneration, bile duct proliferation, and no central veins in the areas of nodular regeneration. It is uncertain whether this was ordinary infectious hepatitis or the early phase of acute yellow atrophy of the liver seen infrequently in thyrotoxicosis.9, 12, 31, 43

7.12 Toxic pancytopenia (1 case)

The patient, a 16-year-old primigravida with folic acid anemia, had multiple trace urine albumin tests and acknowledged her poor compliance with her folate prescription. She delivered spontaneously in the forty-second week of pregnancy under general anesthesia involving nitrous oxide and thiamylal. The hematocrit level fell from 25% to 18%, and hemoglobin decreased from 13.2 to 6.7 gm/dl. A bleeding diathesis developed, and 5 U of platelets were given during labor. Hypersegmentation of polymorphonuclear leukocytes was prominent. Postpartum the course was marked by gross hematuria, sepsis, and pancytopenia (2000 white blood cells/mm3). The terminal event was occasioned by adult respiratory distress syndrome and brain death for 4 days before somatic death 12 days postpartum. The unifying component of this case is the further suppression of vitamin B₁₂ and folate metabolism by nitrous oxide anesthesia.8.41

7.13 Unclassified (I case)

The patient was a 29-year-old gravida 8, para 6, abortus 1, carrying triplets, which all delivered spontaneously at 371/2 weeks under light inhalant anesthesia. The triplets weighed 2268, 1814, and 2041 gm in birth order. All three survived. The mother died 48 days postpartum after an uncertain period of an illness characterized by constant vomiting. There was a possible episode of vomitus aspiration. A local medical examiner viewed the body but did not perform an autopsy, leaving unknown the nature of this unobserved puerperal death.

8. Pathologic investigation of maternal deaths

It is ironic, but not unexpected, that the one case unclassified out of a series of 281 maternal deaths (Section 7.13) was one in which an autopsy would have been of great material assistance in finding an explanation for the outcome. Several possibilities come to mind, especially to the minds of the working party after their extensive review of this experience. One would be puerperal myocarditis or myocardiopathy. Another, given the season of death, would be influenzal pneumonia. However, the more important point to be made here is that the more information available in every and any case the more likely (1) the death will have a cogent explanation, and (2) the greater will be the contribution of the insights of that case to our understanding of the morbid pathophysiology of pregnancy generally. The notes of the working party reflect from time to time the statement "... really needed an autopsy on this case . . ."

We return to our initial treatment of the definition of maternal mortality, in which we differ from the proposed consensus statement. The actual number of maternal deaths has fallen, not necessarily to an irreducible minimum, but certainly to a gratifyingly lower rate than when Dr. Jewett began his work. With the current societal restraints on costs of medical care and the improvements in obstetric care in particular, more and more parturient women are sent home earlier. In accordance with the obvious, a higher proportion of maternal deaths will occur out of hospital and might possibly seem to fall into the jurisdiction of medical examiners or coroners. It is one purpose of our study and this review to provide such physicians and pathologists with the broadest understanding of the possibilities inherent in a maternal death. With respect to the forensic needs of such cases, few are obviously accidental or criminal. We found eight deaths from vehicular collision and five deaths from homicide. There was only one death in which possible negligence of health care personnel was a factor, and only one other attributed to"probable suicide." Finally, one death occurred after a fall during a serious house fire. Many of these cases are medically and obstetrically complex and "viewing the body" is an insufficiently scientific approach to the needs of the matter. Most cases are medical problems, ones in which the medical profession, in general, and medical examiners, in particular, share a common need to know, which suggests the basis for cooperative enterprise.

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In one sense our society will be judged by the style, character, and direction of the care provided pregnant women, who represent our species at one of the most vulnerable periods of its life span.

Inherent in this point of view is the concomitant need to study each failure, each maternal death, with the fullest measure of present scientific and technical knowledge while expanding our avenues of interest and concern. The ultimate test of professionalism is the practice of dispassionate, comprehensive investigation to which the attending physicians must give as much allegiance as the consulting pathologist and the reviewing committees. To that end, one by which no case is diminished by being ignored, progress to 100% modern autopsy examination is just the first step. The second step is for all physicians and pathologists who might have or gain responsibility for performing and reporting these autopsies to make themselves better informed on the scope and essence of maternal mortality. We hope this report will be viewed as helpful to that

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LETTERS TO THE EDITORS

Gonadotropin-releasing hormone agonist and estrogen-progestogen replacement therapy

To the Editors: We read Barbieri's article (Barbieri RL. Gonadotropin-releasing hormone agonists and estrogen-progestogen replacement therapy. Am J OBSTET GYNECOL 1990;162:593-5) with interest. There are a few points on which we wish to comment.

A number of articles on gonadotropin-releasing hormone (GnRH) agonist treatment and bone metabolism report divergent results influenced by small patient numbers, different doses and routes of administration, and methods and sites of bone loss estimation. Studies^{1, 2} in which the usual 6-month treatment was extended showed further bone mineral density reduction in the spine. That bone mineral density remained significantly below baseline level 6 months after treatment is not surprising because a substantial reduction in bone mineral density has been demonstrated in untreated women before menopause.³

There is no evidence that estrogen replaces lost bone, only that it prevents further loss. There being no long-term follow-up of the effects of GnRH agonist-induced bone mineral density reduction, the safety of protracted treatment must be questioned. In our opinion, treatment should be limited to periods shorter than the 2 years recommended by Barbieri. In the study justifying this proposal, half the patients were treated with estrogen; any patients who lost substantial bone mineral density were removed from the study and treated with estrogen.

Bone-protective roles for progestogen during GnRH agonist—induced hypoestrogenemia were examined in a study⁵ in which GnRH agonist was combined with norethindrone. Vasomotor symptoms were reduced, but bone mineral density of the lumbar spine was significantly reduced; similar results were seen with combined GnRH agonist and medroxyprogesterone acetate.⁶ Patients treated with combined GnRH agonist and medroxyprogesterone acetate with or without conjugated estrogen showed no difference in bone mineral density, but neither did the control groups treated with GnRH agonist alone (Barbieri). In these studies bone mineral density was measured on the appendicular skeleton, which is less sensitive to hypoestrogenemia that the spine.⁴

We agree that a regimen for combined GnRH agonist and steroid hormones is a major goal because many patients have problems associated with cyclic steroid changes. Such a regimen should be simple to administer and should produce a stable and physiologic concentration of steroids without metabolic side effects.

We have used a combination of a depot GnRH agonist (Goserelin: ICI Pharma; 3.6 mg/mo) and a low-dose subcutaneous implant of estradiol (Organon Inc., 25 mg/6 mo), which has been shown to have bone-conserving effects and minimal metabolic disturbance. Fifteen women were treated; the plasma estradiol concentration was stable and within normal midfollicular

phase range. The profiles indicate that this regimen could form the basis of long-term therapy for problems such as premenstrual syndrome, irregular menstrual bleeding, and endometriosis and could also be used as an alternative contraceptive.

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Reply

To the Editors: I am thrilled that my article stimulated Gudmundsson et al. to share their scientific ideas and clinical observations. We completely agree that a major goal of gynecologic endocrinology is to develop combined drug regimens of GnRH analogs plus steroid add-back therapy that would be effective in the treatment of endometriosis and myomas and that would minimize the loss of bone mineral density observed when GnRH analogs are used as a single agent.

Our research group was the first to report the results of a 6-month randomized, placebo-controlled trial of combined GnRH analog plus progestin therapy in the treatment of myomas. A member of our group, Dr. Friedman, was also the first to report the results of a small open clinical trial of long-term (27 months) combined GnRH analog plus estrogen-progestin therapy in the treatment of myomas. The experience gained in these clinical trials prompted us to propose the "estrogen threshold hypothesis." We believe that different organs (endometrium, myometrium, bone) have varying sensitivity to the stimulatory effects of estradiol and progesterone. For example, an estradiol concentration of at least 40 pg/ml may be required to stimulate the

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growth of endometriosis implants. In contrast, an estradiol concentration of approximately 30 pg/ml may be sufficient to prevent partially the bone loss often associated with a severely hypoestrogenic state (estradiol concentration of 10 pg/ml). If the estrogen threshold hypothesis is valid, there may be a "therapeutic window" for estradiol in the range of 25 pg/ml to 35 pg/ml in which estrogen-dependent diseases such as endometriosis are suppressed, but in which cumulative bone loss is minimal.

A critical observation made by Dr. Friedman² is that it may be wise to treat with a GnRH analog alone for approximately 3 months before adding estrogen and progestin to the regimen. With this sequential regimen, the estrogen-dependent disease may be forced into hibernation by the GnRH analog treatment and consequently be less responsive to the estrogen-progestin add-back.

An important observation is that treatment of premenopausal women with GnRH analogs for 6 months produces a 6% loss in lumbar vertebral body trabecular bone density and that most or all of this loss is regained within 6 months after GnRH analog therapy is discontinued.⁴ I believe that these findings support the hypothesis that estradiol and progesterone secreted by the ovary can produce an increase in bone-mineral density after premenopausal women are exposed to a short period of hypoestrogenemia.

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Neurologic versus behavioral neonatal assessment after birth asphyxia

To the Editors: We read with interest the report of a study designed to define the threshold of asphyxia beyond which central nervous system damage becomes manifest (Low JA, Muir DW, Pater EA, Karchmar EJ. The association of intrapartum asphyxia in the mature fetus with newborn behavior. AM J OBSTET GYNECOL 1990;163:1131-5).

While we agree with the authors when they state that a proper examination of the relationship between thresholds of asphyxia and outcome requires an accurate diagnosis of fetal asphyxia and a reliable measure of central nervous system injury, we are concerned by their choice of the Neonatal Behavioral Assessment Scale (NBAS) as the outcome measure for this study. In support of this choice, they quote Tronick and Brazelton, who proposed that behavioral assessment in the newborn period may identify mild dysfunctions that are not apparent from standard neurologic examination. They appear to dismiss standard neurologic examination and state that objective measures of central nervous system injury in the newborn period are not available, in spite of published reports to the contrary.¹⁻⁴

In any event the results of this study completely contradict the proposal of Tronick and Brazelton. There were no significant differences between the results of the NBAS of the asphyxia and control groups. Furthermore, even the NBAS results of the four cases identified as encephalopathy by "clinical record of nurses and physicians" did not significantly differ from those of the controls. Should we infer that behavioral testing with the NBAS is insufficiently sensitive to detect neurologically abnormal infants or that it could not be applied to the sickest infants? Whatever the explanation, conclusions cannot be drawn about the very serious problem of thresholds in birth asphyxia from this study.

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Reply

To the Editors: We welcome the opportunity to respond to the correspondence from Amiel-Tison and Stewart. We are in agreement with them regarding the importance of newborn encephalopathy as an indication of central nervous system insult. Standard neurologic examination is a normal function of our neonatal intensive care unit; this care includes continuous observation of newborn behaviour and tone with documentation of any subtle tonic or clonic seizures.

The incidence of metabolic acidosis at delivery (umbilical artery buffer base < 34 mmol/L) in our center is approximately 2%. There are a number of newborns with biochemical evidence of intrapartum fetal as-

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phyxia who do not have evidence of newborn encephalopathy. In our follow-up studies these children, in relation to a control group, have shown an increased incidence of developmental, particularly motor, delay, as measured by the Bayley Scales of Infant Development at 1 year of age.² This suggests that a fetus may experience an acute intrapartum central nervous system insult caused by asphyxia without manifesting newborn encephalopathy.

Studies in the fetal lamb have demonstrated that the fetus that is subjected to sustained partial hypoxemia has important cardiovascular compensatory mechanisms that will maintain normal cerebral oxygen consumption for some time, although decompensation ultimately will occur with a progressive metabolic acidosis.³ We support the notion that corresponding compensatory mechanisms provide a period of protection for the human fetus. However, there is a threshold of asphyxia beyond which central nervous system injury, namely, motor dysfunction, will occur in the surviving child, and it may be accompanied by mental retardation. This threshold remains to be determined.

In this study the newborns of the asphyxia group, with the exception of one with severe newborn encephalopathy, represent the less severe part of the spectrum of intrapartum fetal asphyxia. Experience with the Brazelton NBAS administered by trained examiners suggested that this test might provide an objective measure of minimal central nervous system injury during the neonatal period. Thus the premise in this study was that the NBAS could be a means of identifying central nervous system injury after intrapartum fetal asphyxia was determined biochemically in these newborns without evidence of newborn encephalopathy.

Amiel-Tison and Stewart are correct in stating that this study does not define the threshold of birth asphyxia that is responsible for the neurologic deficits in children. However, this study suggests but does not prove that biochemically determined fetal asphyxia without associated newborn encephalopathy has not been sufficient to cause central nervous system injury. Future developments of more sensitive objective measures of newborn neuropathologic conditions may determine that the NBAS has been insufficiently sensitive to detect minimal central nervous system injury caused by asphyxia.

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CA 125 in breast milk

To the Editors: With great interest I read the article by Hardardottir et al. (Hardardottir H, Parmley, TH II, Quirk JG Jr, Sanders MM, Miller FC, O'Brien TJ. Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm. Am J Obstet Gynecol 1990;163:1925-31) describing the expression of CA 125 with immunohistochemical techniques in embryonic tissues and mammary glands. Furthermore, large amounts of CA 125 in colostrum and milk probes were reported.

To my knowledge the data on CA 125 levels in the colostrum and breast milk need to be analyzed further: Declining concentrations of CA 125 in human milk and serum post partum were published earlier. In this study, on the day of parturition we demonstrated elevations of CA 125 in the maternal sera of all patients. Thus an antigen transfer from the maternal bloodstream into the breast milk must be considered.

Hardardottir et al. failed to describe concomitantly measured maternal CA 125 serum concentrations; therefore a contribution of these values to the colostrum and milk levels must be emphasized.

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REFERENCE

1. Fuith LC, Daxenbichler G, Marth C. CA 125 in human milk and serum. Gynecol Obstet Invest 1989;28:1-3.

Reply

To the Editors: Recently we described the distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm. Using immunohistochemical techniques we observed neticeable staining in the adult apocrine sweat glands and mammary glands. Antigen in frozen sections of lactating breast tissue was detected by the antibodies OC 125 and M11, which recognize different epitopes on the CA 125–carrying glycoprotein. The antigen was found to be localized intraductally in the breast, as opposed to intracellularly. Because of the intraductal localization of the antigen, it was anticipated that breast milk might contain significant concentrations of antigen.

Previously Fuith et al.¹ reported ranges of CA 125 antigen levels in breast milk from 82 to 357 U/ml at 1 week after delivery and 25 to 308 U/ml at 6 weeks after delivery. We observed very high levels of antigen (15,000 to 60,000 U/ml in colostrum; 130 to 1788 U/ml in breast milk from 3 to 7 days post partum and 28 to 203 U/ml 5 to 25 weeks post partum). It also has been reported that relatively moderate increases in serum CA 125 occur after delivery (48 to 500 U/ml). Fuith et al.¹ and Itahashi et al.² attributed these transient elevations of serum CA 125 levels to infusion of antigen into the maternal circulation as a result of placental separation. We believe this is a plausible explanation. The data we present regarding very high levels of CA 125 in colostrum and the fact that antigen is

localized in the intraductal space of the mammary gland would support the notion that the milk antigen may be synthesized and secreted by the breast, just as antigen appears to be synthesized and secreted by both the apocrine sweat glands and the endometrial glands. Leakage of this antigen into the maternal circulation, thereby contributing to the postpartum serum levels, is possible but unlikely unless access of the CA 125 antigen through the endothelium to the circulation can be accounted for. The likelihood that serum antigen is the source of the CA 125 in colostrum also is unlikely because of the great disparity in concentrations between serum CA 125 and colostrum CA 125. Nonetheless, we believe that paired concurrent assays of CA 125 in colostrum and serum in the postpartum period would be useful in the support or denial of these conclusions.

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Risk of open spina bifida

To the Editors: Genetic counselors try to provide couples with the best estimate of their risk of birth defects. With the implementation of maternal serum α -fetoprotein (AFP) screening programs, patients with elevated maternal serum AFP levels are referred to ultrasonography specialists to look for fetal open spina bifida and other lesions. Ultrasonographic examinations will show most of these patients to have normal fetuses. The revised numerical risk of a fetus having open spina bifida calculated on the basis of its mother's maternal serum AFP level plus normal results on ultrasonographic examination is important information that patients may want to use in deciding whether to accept the risk of amniocentesis. The figures presented by Thornton et al. (Thornton JG, Lilford RJ, Newcombe RG. Tables for estimation of individual risks of fetal neural tube and ventral wall defects, incorporating prior probability, maternal serum α -fetoprotein levels, and ultrasonographic examination results. Am J OB-STET GYNECOL 1991;164:154-60) are therefore potentially very useful.

My initial inspection of Tables II through IV focused on the upper of the three lines for each particular maternal serum AFP level. Each figure in this line "assumes that ultrasonography provides no information beyond maternal serum AFP" level. In other words, I presume the risk would be calculated solely on the basis of the maternal serum AFP level.

If the population incidence of open spina bifida is 1 in 1000, my reading of the tables indicates that a patient with a maternal serum AFP level of 2.0 multiples of

the median would have a risk of fetal open spina bifida of 1 in 1800. If the ultrasonographic information is excluded, how can a patient with an *elevated* maternal serum AFP level end up with a *lower* risk than the risk that existed before the screening test was performed?

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Reply

To the Editors: I thank Dr. Hershey for his interest in our paper. At first sight it is indeed surprising that a woman with a maternal serum AFP level of 2.0 multiples of the median should have a lower risk of open spina bifida than if she had had no test at all. It is nevertheless correct. The posterior odds of open spina bifida are increased only when the maternal serum AFP likelihood ratio rises above unity. This happens when the curves of the maternal serum AFP probability distributions for affected and unaffected pregnancies cross. Fig. 1 in our article, a hand-drawn plot of the probability distributions, was only intended to illustrate the derivation of the likelihood ratio; the curves were drawn (inaccurately) to cross at 2.0 multiples of the median. The curves should cross at a maternal serum AFP level between 2.0 and 2.5 multiples of the median. Nevertheless it is easy to see from Fig. 1 that a maternal serum AFP level of 1.5 multiples of the median gives a likelihood ratio of <1.0; if the curves were drawn correctly, it would be clear that the likelihood ratio for a maternal serum AFP level of 2.0 multiples of the median is also <1.0.

The assumption that the risk of open spina bifida automatically increases if the maternal serum AFP is >1.0 multiples of the median is an error resulting from intuitionist thought. Dr. Hershey used the "representativeness" heuristic to calculate risk¹; because raised levels of maternal serum AFP are "representative" of open spina bifida, he assumed that the risk was raised. The truth is sometimes counterintuitive, but our tables should prevent this mistake in future.

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Postnatal outcome depends on prenatal history

To the Editors: We read attentively the article by Lipper et al. (Lipper EG, Ross GS, Auld PAM, Glassman MB. Survival and outcome of infants weighing <800 grams at birth. Am J OBSTET GYNECOL 1990;163:146-50), and we wish to express the opinion that this study has no scientific meaning.

In particular, the weight of the fetus at birth is for obstetricians a topic of great importance and interest, and we should not forget to differentiate the weight of an appropriate-for-gestational-age newborn from the

weight of a growth-retarded fetus. All of the modern literature differentiates the postnatal outcome according to the prenatal history of the fetus.

The study of Lipper et al. brings confusion at a time when pediatrics and obstetrics are beginning to agree on the definitions of the terms.

A knowledge of the outcome of a fetus <800 gm is of no utility if we are not acquainted with the gestational period in which the infant was born.

Your JOURNAL usually is rightly critical in selecting studies coming from non-English-speaking authors, and we are astonished that it publishes with such simplicity data so barely scientific.

Claudio Giorlandino, MD

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Reply

To the Editors: Although it is an important practice in neonatology to differentiate appropriate-for-gestational-age from small-for-gestational-age infants, our study only reported on survival and outcome of infants weighing between 500 and 799 gm at birth whose mean gestational age was 25.5 ± 1.8 weeks (see Table I of our article). Within this narrow range of birth weight and gestational age intrauterine growth retardation could not be a factor relating to outcome, as suggested by Dr. Giorlandino.

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Anticomplementary activity in serum and abortion

To the Editors: We read with interest the article by Quinn and Petric (Quinn PA, Petric M. Anticomplementary activity in serum of women with a history of recurrent pregnancy loss. Am J Obstet Gynecol 1988;158:368-72). We would like to present our similar study of habitual abortions, preeclampsia, spontaneous abortions, endometriosis, and normal pregnancies. We performed complement fixation tests with cardiolipin antigen and tested the anticomplementary activity of the sera of these patients. Cardiolipin is a negatively charged phos-

pholipid abundant in mitochondrial membranes. The mitochondria are one type of activator of the complement cascade through the classic pathway.¹

Autoantibodies against cardiolipin cause extensive placental thrombosis and subsequent fetal losses. 2.3 Anticardiolipin antibodies have been detected in recurrent fetal losses, preeclampsia, and endometriosis. Thus it is possible for antibodies that form immune complexes with cardiolipin to activate the complement system and have an anticomplementary effect, as was shown by Quinn and Petric.

We had 50 patients with histories of habitual abortion (three or more abortions), 22 patients with histories of spontaneous abortion (fewer than three abortions), 6 patients with histories of late intrauterine fetal death, 26 patients with histories of severe preeclampsia, and 58 patients with histories of endometriosis. As control groups we had 26 women with histories of uneventful gestations and deliveries, 28 prepubertal girls, and 10 men. Anticomplementary activity was found in 19 (38%) of the 50 women with habitual abortions, in 4 (18%) of the 22 women with spontaneous abortions, in 7 (27%) of the 26 women with preeclampsia, and in 13 (22%) of 58 women with endometriosis. None of the patients who had had intrauterine deaths showed anticomplementary activity. Only 2 of 28 serum samples in the prepubertal-girls control group showed anticomplementary activity. Anticardiolipin antibody tests were done in all of the groups with enzyme-linked immunoadsorbent assay. The results of the serologic tests are shown in Table I.

Autoimmune abnormalities are thought to have a role in the pathogenesis of these disorders. There are two possible explanations for the anticomplementary activity seen. First, immune complexes or antigens such as cardiolipin can nonspecifically fixate complement and cause an anticomplementary effect. Second, patients with circulating immune complexes can also have autoantibodies against complement components.

Sibel Ergüven, PhD, and Ekrem Gülmezoğlu, MD Department of Microbiology, Hacettepe University, Ankara, Turkey A. Metin Gülmezoğlu, MD

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Table I. Serologic tests in patient and control groups

•		Anticardiolipin				
	IgG+		IgM+		 	
	No.	%	No.	%	Anticomplementary activity	
Habitual abortion	10	20	. 8	16	19 38.0	
Spontaneous abortion	4	18.2	4	18.2	4 18.2	
Intrauterine death	1.	16.6	. 3			
Severe preeclampsia	1	3.8	3	11.5	7 26.9	
Endometriosis	. 9	15.5	1	1.7	13 22.4	
Normal pregnancy and delivery	2	7.7	2	7.7	Makes Makes	
Prepubertal girls				_ ' '	2 7.1	
Men .		'		· · · <u>-</u>	*******	

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Response declined

Fetal hiccups and the umbilical ring

To the Editors: DeLee stated that fetal hiccups is "one of the most interesting phenomena of intrauterine life."1 To date, no clearly defined mechanism has explained this fetal behavior. Pillai and James² recently summarized that fetal hiccups become noticeable at about 24 weeks' gestation and diminish toward term. They described fetal diaphragmatic movements changing character and becoming more cyclic with increasing gestational age; they suggested that the fetal breathing center may be different from the mechanism that controls hiccups. The nerve impulses that may cause diaphragmatic contractions more plausibly originate by way of the umbilical ring. Cholinergic and adrenergic nerve terminals or end nests have been identified in the umbilical cord 20 cm out and concentrated toward the umbilical ring.^{3, 4} These nerve fibers run with the umbilical vein and sacral plexus to join the phrenic ganglion and celiac ganglion in proximity to the ductus venosus.5,6 These fibers run with the vagal trunks and eventually may interact with the phrenic nerve by way of the medulla and respiratory center. The phrenic nerve sends fibers to the pericardium, phrenic ganglion, sympathetic plexus, and hepatic plexus. Compression or stretch of the umbilical cord may lead to spasm of the ductus venosus7 or contractions of the . diaphragm via a similar reflex arc. As has been suggested by Ellison,3 the umbilical ring may function before birth rather than after birth and therefore is difficult to study once the infant is born. With this in mind it can also be theorized that the ductus arteriosus and foramen ovale are linked to the umbilical ring. Two cases of cord entanglement with fetal heart rate variables were noted in which the fetuses had daily hiccups near 36 weeks' gestation. Three to four episodes per day were recorded by the mothers for several weeks, along with fetal movements. The possibility of detecting cord entanglement or compression may be specific to persistent fetal hiccups.

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Patients with preeclampsia may be insensitive to atrial natriuretic factor

To the Editors: August et al. are disturbed by the apparent discrepancy that increased blood atrial natriuretic factor is associated with relative hypovolemia in women with preeclampsia (August P, Lenz T, Ales KL, et al. Longitudinal study of the renin-angiotensinal dosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia. Am J Obstet Gynecol. 1990;163:1612-21). For a solution, they challenge the hypovolemia concept, although it has been confirmed by many different studies with techniques other than Evans blue dye dilution. ^{1,2} Unfortunately, there is no currently approved technique for measuring plasma volume in pregnant women to re-prove the concept.

The apparent discrepancy between the high level of atrial natriuretic factor found in many studies of women with preeclampsia and their *apparent* hypovolemia can be explained by preeclampsia causing insensitivity to atrial natriuretic factor. Such an insensitivity could explain the hypertensive and edema symptoms of preeclampsia.⁵

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Response declined

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby—Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

The Second International Symposium on Gynecologic Surgery and Adhesion Prevention, January 31–February 2, 1992, the Ocean Grand Hotel, Palm Beach, Florida. For further information/formal brochure, contact Symposia Medicus, 1299 Newell Hill Place, Suite 301, Walnut Creek, CA 94596-5220. Tel.: (415) 935-7889.

1992 Roussel Prize. The ever-increasing importance of steroids in man, animal, and, perhaps in the future, plant health provided the incentive for the establishment of an International prize to support basic research in this area. The Roussel Prize, created in 1968 at the instigation of Prof. J. Mathieu, is awarded every two years to one or two chemists, biochemists, or physiologists whose work has been selected as the most outstanding by an international jury of distinguished scientists. The next Roussel Prize (\$40,000), which is scheduled in Autumn, 1992, is intended to reward one or more researchers for outstanding work on steroids and other squalenoids published before Dec. 31, 1991. The composition of the Jury for the year 1992 is as follows: President: Sir Derek Barton. Members: Professors M. Akhtar, J. Gorski, N. Ikekawa, J. Mathieu, Y. Mazur, and J. Sjövall. Candidates for the Prize may be of any nationality and from any laboratory. Nominations should be put forward by a person of high scientific standing, by means of the appropriate form and addressed to the President of the Jury or to the Secretariat of the Roussel Prize, before Jan. 1, 1992. Forms and any further information may be obtained from the Secretariat on request: Secretariat of the Roussel Prize, Institut Scientifique Roussel, 35 Boulevard des Invalides, 75007 Paris, France.

Division of Neonatal Medicine, University of California Irvine Medical Center, "The New Decade: Neonatal/Perinatal Medicine," Alaskan Cruise Conference, September 8-15, 1992. For further information contact William G. Cvetnic, MD, or Terry Pliska, RN, Division of Neonatal Medicine, University of California Irvine Medical Center, PO Box 8119, Orange, CA 92664-8119. Tel.: (714)634-6933.

Common Problems in Obstetric Care II, April 20-25, 1992, Westin Maui, Maui, Hawaii. Sponsored by the Departments of Obstetrics and Gynecology, Cornell University Medical College—The New York Hospital and The University of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3059.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1991 issues from the Publisher, at a cost of \$61.00 for domestic, \$88.27 for Canada, and \$84.00 for international for Vol. 164 (January-June) and Vol. 165 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. Payment must accompany all orders. Contact Mosby—Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, USA; phone (800) 325-4177, ext. 4351.

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PERINATOLOGIST

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Send C.V. to:

Search Committee
c/o Millard Simmons, M.D.
Chief, Division of Maternal Fetal Medicine
Department of Obstetrics and Gynecology
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Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone J Reprod Med 1989;34(8):540-542 Please see complete Prescribing Information a brief summary of which is on the preceding

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SUPPLEMENT TO

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VULVOVAGINITIS: CAUSES AND THERAPIES

Bethesda, Maryland February 4 and 5, 1991

Moderators

Florence P. Haseltine, PhD, MD Benson J. Horowitz, MD



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Moderators

Florence P. Haseltine, PhD, MD Director, Center for Population Research National Institute of Child Health and Human Development Rockville, Maryland

Benson J. Horowitz, MD
Associate Clinical Professor of Obstetrics/Gynecology
University of Connecticut School of Medicine
Chairman, Vaginitis Section
International Society for the Study of Vulvar Disease
Hartford, Connecticut

Proceedings of a National Institutes of Health Conference sponsored by the National Institute of Child Health and Human Development and the International Society for the Study of Vulvar Disease

Supported in part by an educational grant from ORTHO PHARMACEUTICAL CORPORATION

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Kenneth D. Hatch

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American Journal of Obstetrics and Gynecology

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October Part 2

VULVOVAGINITIS: CAUSES AND THERAPIES

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Hartford, Connecticut

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Sam A. Nixon, MD

Houston, Texas

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The vaginal ecosystem

Per-Anders Mårdh, MD

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The ecology of vaginal flora is highly susceptible to numerous endogenous and exogenous influences. Possible mechanisms that might influence changes in the vaginal flora are microbial antibiosis, adhesion to the vaginal epithelial lining, e.g., availability of receptors, hormonal changes including physiologic alterations, disease states, drug therapy, sexual activity, and to a lesser extent, the immunologic status of the person. The role that these mechanisms may play in promoting vaginitis, vaginosis, and possibly pelvic inflammatory disease is explored. (AM J OBSTET GYNECOL 1991;165:1163-8.)

Key words: Vaginal flora, microbial ecology, vaginitis, vaginosis, etiology

During recent years the ecology of the vagina has been studied in both healthy women and those with pathologic conditions, such as bacterial vaginosis, candidiasis, and trichomoniasis. The microbial ecology of the vagina is a very delicate system that can be easily altered. Recent research has not only unveiled modes by which the vaginal flora and epithelial lining are changed by altered host-parasite interactions, but ways in which endogenous and exogenous factors such as hormonal therapy and other iatrogenic manipulations such as antibiotic therapy can affect the composition of this flora (Table I).

Vaginitis versus vaginosis

Vaginal infections, such as *Trichomonas vaginalis* and candidiasis, generally induce an inflammatory response in the vaginal wall, which is usually accompanied by an increased number of leukocytes in the vaginal fluid. Such an inflammatory response is the hallmark of "itis" conditions. An inflammatory response of the lower female genital tract may also accompany viral infections caused by herpes simplex and human papillomavirus.

Bacterial vaginosis (BV) accompanied by a vaginal flora change is not characterized by an inflammatory response. BV has alternatively been referred to as "nonspecific" vaginitis and vaginal bacteriosis. Lactobacillinduced cytolytic vaginosis (also called Döderlein cytolysis) is another form of vaginosis in which leukocytes are sparse.

Vaginal fluid

Vaginal fluid may contain various portions of cervical secretion, uterine, follicular, and peritoneal fluid, as

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Table I. Etiology of changes in vaginal flora

- Therapy with antibiotics, cytostatics, corticosteroids, antivirals, antifungals, and irradiation
- Vaginal douching
- Malformation and anatomic deformity after surgery and/or irradiation
- Cysts, hymen, polyps
- Immunosuppressive conditions, e.g., AIDS (less common)
- Hormonal changes from aging, use of oral contraceptives (less common), or therapy for disease
- Uncontrolled diabetes
- Foreign bodies, e.g., intrauterine device, or retained tampon or diaphragm
- Spermicides

AIDS, Acquired immunodeficiency syndrome.

well as exfoliated epithelial cells, bacteria, and bacterial products. Occasionally semen, contraceptive and hygiene products are added to the vaginal contents.

Composition of vaginal flora

"Normal" vaginal flora is usually dominated by lactobacilli. In contrast, the vaginal flora of women with BV consists of a combination of aerobic, facultatively anaerobic, oxygen-tolerant anaerobic, and strictly anaerobic bacteria. ^{1,2} BV in particular is associated with a high number of organisms, including *Gardnerella vaginalis* (the "clue" cell organism), *Bacteroides bivius* and other *Bacteroides* species, *Mycoplasma hominis*, and the newly named species *Mobiluncus mulieris* and *Mobiluncus curtisii*.

G. vaginalis can also be found in many healthy women, as can M. hominis. The latter organism occurs in approximately 15% to 30% of healthy, sexually active women. M. hominis and G. vaginalis can also be part of the vaginal flora in women with trichomoniasis or candidiasis, but the two Mobilincus species are rarely found in these two other conditions.

A strong case for classifying M. hominis, traditionally

Table II. Inhibition of lactobacilli by different vaginal isolates

,	No. of		No. of strains inhibiting	· ·
Species	No. of strains	L. acidophilus	L. jensenii	L. fermentum
G. vaginalis	. 10	4	3	. 4
B. bivius	. 2	1	0 .	0
B. disiens	3	. 1	0	. 0
M. curtisii	10	2	0	1
Peptostreptococcus spp.	5	2	1	2

Modified from Nagy, Petterson, Mårdh PA. Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. APMIS (Copenhagen) 1991;99:739-44.

Table III. Number of H₂O₂- producing lactobacilli isolated from women with and without signs of BV

	No. of H ₂ O ₂ -producing strains				
Lactobacillus species	With BV	Without BV			
L. acidophilus	5/11	17/20			
L. jensenii	2/7	8/11			
L. fermentum	0/2	2/3			
L. delbruckii	2/4	2/2			
L. leichmanii	0/3	2/2			
L. salivarius	0/1	3/4			
L. brevis	0/1	0/1			
L. casei	0/7	1/2			
L. cataneforme	0/1	0/0			
Lactobacillus species	0/2	2/2			
Total	9/39 (23%) 37/47 (78%)			

Modified from Nagy, Petterson, Mårdh PA. Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. APMIS (Copenhagen) 1991;99:739-44.

considered a sexually-transmitted disease organism, as a BV-associated organism can be made based on, among others, the following observations: (1) It occurs in a high proportion of women with BV but only rarely in their sexual partners³; (2) it can be isolated from bladder urine, similar to G. vaginalis and M. curtisii, both BV-associated organisms³; (3) M. hominis can be isolated from extragenital abscesses in persons without signs of BV and can cause postpartum bacteremia like the latter two organisms; and (4) like G. vaginalis, it may occur in the intestinal flora of healthy men and women.

M. mulieris is almost exclusively found in the vagina of patients with BV. M. curtisii has been recovered from the upper genital tract in women with pelvic inflammatory disease.² In an ongoing Swedish study (the Afa study) of women attending a contraceptive clinic, 12.6% of 632 women had BV, the diagnosis of which was based on the presence of three of the four following criteria: (1) gray, homogenous vaginal discharge, (2) vaginal pH >4.7, (3) a positive amine test, or (4) a moderate or greater number of "clue" cells. This number increased to approximately 25% when less than a moderate number of "clue" cells were included.

Characteristic of Mobiluncus bacteria is corkscrew-like

motility, seen in approximately 16% of the women in the Afa study.

In contrast to *Mobiluncus* species, *Bacteroides* species, anaerobic gram-positive cocci, and fusobacteria are found with equal frequency in BV, trichomoniasis, and candidiasis.¹

Campylobacter fetus, subspecies venerealis, is an organism relatively recently discovered in the vaginas of healthy women and those with BV. It is known as a cause of abortion in animals but not in women.

The intestinal tract as probable microbial reservoir

It is currently believed that the intestinal tract is the reservoir for organisms found in the vagina of women with BV. BV-associated organisms, such as *Mobiluncus* species, can be found in the rectum of women with BV as often as those without BV. They are also isolated from the intestinal tract of men without BV-affected partners and in fecal specimens of children.

Mechanisms involved in vaginal flora alterations

Hormonal factors probably play a role in the pathogenesis of BV, because it is a condition that affects only women in reproductive ages. However, oral contraceptives do not affect its incidence. One other hypothesis is that BV is the result of altered vaginal antibiosis or antagonism between organisms occurring in the vagina.

Lactobacilli decrease the vaginal pH through production of acidic products, thereby making the vagina inhospitable to some bacterial species. The efficiency of antibiosis is influenced by the pH. However, the presence of certain anaerobic lactobacilli in the vagina of women with BV does not afford the same protective antibiosis that facultatively anaerobic lactobacilli do. It is now believed that the antibiosis exhibited by some lactobacilli may, among other mechanisms, be from endopeptidase production as indicated by in vitro studies. The vaginal flora of lactobacilli, which is dominated by Lactobacillus acidophilus (Table II), is itself regulated by lactocins, which are bacteriocin-like products of lactobacilli and directed against lactobacilli.

Another mechanism for regulating the ecology of the vagina may be the production of hydrogen peroxide

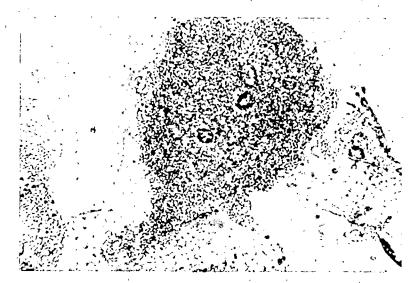


Fig. 1. Typical "clue" cell of bacterial vaginosis, Gardnerella vaginalis attached to vaginal epithelial

(H₂O₂) by lactobacilli, because healthy women are more likely to have H₂O₂-producing lactobacilli than women with BV (Table III).

Candicidin, like bacteriocins and lactocins, may be involved in the regulation of the occurrence of yeast fungi in the vaginal flora.

Receptor availability and binding factors

Alterations in the composition of the vaginal flora that facilitate bacterial colonization may also be related to changes in the availability of receptors on eukaryotic cells of the vaginal epithelial lining. It is likely that physiologic changes of the vaginal milieu are often the result of endogenous rather than exogenous factors. The chemical nature of receptors for G. vaginalis, Candida albicans, and the two Mobiluncus species are of carbohydrate nature.

Whether there is a change in the glycocalyx covering of the epithelial cells or changed exposure of the receptor sites that facilitates bacterial adherence is not yet clear. Increased pH levels are generally associated with an increased ability of bacterial binding to eukaryotic cells; it is not known whether the change in pH facilitates a change in vaginal flora or vice versa.

One striking feature of BV is the occurrence of "clue" cells,7 that is, exfoliated vaginal epithelial cells covered by G. vaginalis bacteria (Fig. 1). These cells could also have been referred to as "glue" cells because the bacteria appear to be glued to the epithelial cells. Likewise, recent microbiologic studies have revealed that the curved rod of Mobiluncus (Fig. 2) attaches to vaginal epithelial cells, creating "comma cells."

Among other possible factors that may be involved in bacteria persisting in the vagina are mucus including glycoproteins, lactoferrin, and metal ions, such as zinc and magnesium. Lysozyme, which is found in most

body fluids and is primarily active against gram-positive organisms, interferes with the ability of bacteria but not with chlamydiae to form inclusions in tissue cell cultures.

Fibronectin, a glycoprotein found in connective tissue and free in some body fluids, including vaginal fluid, may also play a role in the persistence in the vagina of organisms such as staphylococci, streptococci, and lactobacilli.

Lactobacilli and other bacteria may be randomly distributed on the surface of epithelial cells, which should not be confused with bacteria attached to cell surface receptors or glycocalyx components. There might be receptor analogues in the glycocalyx to which bacteria may associate.

Hormonal changes

It is well known that throughout a woman's life, physiologic hormonal changes alter the vaginal flora. One of the prime examples is pregnancy, during which the vaginal pH generally becomes more acidic. Pregnancy increases the risk of vaginal candidiasis. This fact suggests that pH alterations and cellular changes may play a role in regulating overgrowth of Candida. A hormonal influence is also suggested to explain cytolytic vaginosis, which generally occurs in young girls with an abundant overgrowth of lactobacilli and lysis of epithelial white blood cells.8

Oral contraceptives or other steroid treatment can also theoretically alter the vaginal flora. Modern oral contraceptives have generally little influence on the vaginal flora. One observation of interest in this conjecture is that there is evidence of estrogen receptors on C. albicans organisms. However, its role in regulating the occurrence of this yeast fungi in the vagina has not been borne out by clinical experience.

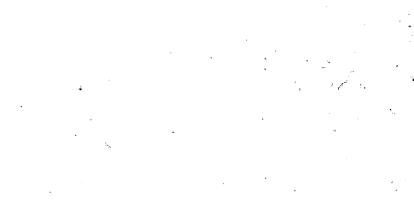


Fig. 2. Typical "comma"-shaped cell seen in bacterial vaginosis caused by the curved rod-shaped *Mobiliuncus* species.

Table IV. Number of lifetime sexual partners of women with and without signs of BV*

	. 0	
No. of partners	No. with signs of BV, $N = 78$ (%)	No. without signs of BV, N = 556 (%)
1 .	11 (16)	86 (15)
2-3	14 (24)	135 (24)
4-5	13 (21)	119 (21)
6-10	14 (23)	130 (23)
11-20	20 (12)	64 (13)
21-49	3 (3)	18 (3)
50-100	3 (0.5)	3 (1)
>100	0 (0.2)	1 (0.2)

Modified from Kallings, Brihmer, Sikström, Mårdh. Bacterial vaginosis in women attending for contraceptive advice. Unpublished data.

*Defined as fulfilling three of four of: vaginal pH >4.7, positive amine test, gray homogenous discharge, and moderate to high number of "clue" cells.

Vaginal contraceptives that may influence the vaginal ecology

The vaginal flora can be altered by prolonged retention of a diaphragm and tampon and by vaginal douching. Moreover, the use of an intrauterine device, particularly one with a "tail" extending through the cervical os, increases the risk of pelvic inflammatory disease in women with BV flora. 9, 10

Spermicides containing nonoxynol-9 may affect the vaginal flora. Their use in the form of a cream or in a contraceptive sponge is associated with an increased resistance to some sexually transmitted diseases, such as gonorrhea, chlamydial infection, and human immunodeficiency virus infections.^{11, 12} An in vitro effect of spermicides against the agents causing these infections can be shown.

Disease states

Some generalized disease states, such as poorly controlled diabetes mellitus, appear to predispose women to vaginal candidiasis.

Although an increased incidence of oral and intestinal candidiasis is generally more common in patients with acquired immunodeficiency syndrome than in comparison groups, this does not seem to hold true for the genital tract.

Drug therapy

When the endogenous vaginal flora are eradicated by broad-spectrum antibiotics, such as during the treatment of sexually transmitted diseases or respiratory tract infections, overgrowth of *Candida* (generally *C. albicans*) can be anticipated in a subpopulation of women. Long-term therapy, however, is more likely to cause this type of overgrowth than short-term treatment. Likewise, long-term cytostatic, corticosteroid, antiviral, and antifungal drug therapy can also alter the vaginal flora, thus promoting vaginitis.

The role of the male sex partner

Although the male sex partner plays an essential role in the pathogenesis of sexually transmitted disease-causing cervicitis and in vaginal infections such as in trichomoniasis, herpes, and genital warts, the role of the partner in the pathogenesis in BV and candidiasis is less certain.

Colonization with BV-associated bacteria is no more frequent in the urethra of sex partners of women with BV than it is in the sex partners of women without BV.¹³ In uncircumcised male partners of women with BV, a higher colonization rate is found in the sulcus of the glans penis than in the urethra.

In a study of 100 men at a sexually transmitted disease clinic, M. curtisii and G. vaginalis were isolated in approximately 10% of patients, whereas M. mulieris was never recovered.14 In another study, similar isolation rates were found in male partners of women with BV, but only 5% of men, independent of whether their sex partners had BV, were colonized rectally with BV-associated bacteria.11 These observations argue against sexual transfer being important and that concomitant treatment of women with BV and their male sex partners with metronidazole has any major impact on BV.14 Although one study found that the use of condoms may normalize the vaginal flora in women with BV,15 for the most part, the evidence suggests that BV is not a sexually transmitted disease. This argument is strengthened by the recent finding that the prevalence of BV does not increase by an increasing number of lifetime sexual partners (Table IV).

In women with vaginal candidiasis, male partners, particularly uncircumcised ones, are often colonized by C. albicans. A small proportion of these men develop balanitis. In isolated cases, concomitant treatment of both partners may be useful, and use of condoms during intercourse may protect women from recurrent bouts of candidiasis. Moreover, yeast colonization in the male partner can also disappear once the woman alone has been treated. Yet, sexual transmission of candidiasis resulting in clinical disease is by no means commonplace.

Local immune defense

The secretory antibody defense mechanisms, which agglutinate bacteria, do not seem to be highly efficient in preventing BV and other types of changes in the vaginal flora. This is possibly because of bacterial proteinases produced by vaginal colonizers, many of which have the ability to break down IgA-1.

The role of IgG antibodies and complement factors in BV and candidiasis in microbial cell lysis has not been well studied, but it appears that IgG antibody opsonization of bacteria does not occur very efficiently in vaginal fluid. The level of vaginal fluid complement is only 11% of that in serum. Moreover, the effect of complement may be inhibited by semen, which also has additional inhibitory effects on the immune system in the genital tract. The inefficiency of the immune system to hinder infections of the lower genital tract may be exemplified by trichomoniasis. Although humoral IgM, IgG, and IgA antibodies to T vaginalis and IgG and IgA antibodies can be detected in vaginal fluid, these antibodies do not seem to play any protective role in this type of vaginitis. A T-cell response can also be demonstrated in trichomoniasis, but without any great effectiveness in protecting the individual from developing this condition.

Long-term consequences of vaginitis and vaginosis

Organisms that colonize the vagina in women with BV, in particular, organisms belonging to the genus Mobiluncus, can spread to the upper genital tract and be associated with pelvic inflammatory disease, that is, endometritis or salpingitis.2 It is also a well-established fact that infections of the lower genital tract are correlated with dysplasia and cervical neoplasia. The most striking data are for certain types of human papillomavirus, which has been reported to be associated with neoplastic cervical changes.16 Although similar observations have been made for M. hominis¹⁷ and Mobiluncus species, but few if any of these studies claiming an association between human papillomavirus and neoplasia have controlled for the vast number of organisms present in the genital tract of carriers of human papillomavirus. The oncogenic potential of nitrosamines, which are produced by certain vaginal bacteria inhabitants of women with BV, has also been brought forward.

Finally, the possibility that vaginitis or vaginosis may increase the possibility of acquiring or transmitting acquired immunodeficiency syndrome needs to be studied. Such an association has already been demonstrated for genital lesions caused by Hemophilus ducreyi18 and by C. trachomatis.19

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Epidemiology of vaginitis

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Vaginitis is one of the most common problems in clinical medicine, and it is the reason cited most often for visits to obstetricians and gynecologists. This article reviews the epidemiology in the United States and Scandinavia for the three major causes of vaginitis: candidiasis, trichomoniasis, and bacterial vaginosis. The incidence of candidiasis has increased dramatically during the past decade, with an increase in the percentage of non-albicans Candida strains. However, in Scandinavia the incidence of candidiasis has been relatively stable, between 10% and 30%, during the past 5 years. The incidence of *Trichomonas* has decreased dramatically in both the United States and Scandinavia during the past 15 years, partly attributable to the advent of metronidazole. In the United States bacterial vaginosis continues to be the leading variety of vaginal infection, affecting a broader spectrum of women than gonorrhea. The prevalence of bacterial vaginosis in Scandinavia is about 30%, and this percentage increases with age according to studies of patients at sexually transmitted disease clinics. (AM J OBSTET GYNECOL 1991;165:1168-76.)

Key words: Vaginitis, vulvovaginal candidiasis, trichomoniasis, bacterial vaginosis

In the recent past, the problem of vaginitis has all too often been ignored by the medical community or regarded merely as a minor annoyance to women. Perhaps the late Herman Gardner put it most eloquently when he stated, "Vaginitis must cause more unhappiness on earth than any other gynecologic disease. In addition to the many physical and emotional problems associated with vaginitis, the economic loss involved is of astronomic proportions."

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When discussing the problem of acute vaginitis, we must be aware of the following facts:

- Vaginitis is one of the most common problems in clinical medicine.
- Vaginitis accounts for more than 10 million office visits each year.^{2, 3}
- Vaginitis is the most common reason for a patient to visit her obstetrician-gynecologist.
- Vaginitis is found in 28% of women attending sexually transmitted disease (STD) clinics.
- Candidiasis is the second most frequent vaginal infection in the United States and the primary vaginal infection in Europe.
- Twenty-five percent to 40% of women with an

overgrowth of vaginal Candida are asymptomatic carriers.

- Mild leukorrhea resulting from Candida may be dismissed as a physiologic condition.
- Vaginitis may produce symptoms similar to urinary tract infections (dysuria).
- Vaginal discharge is among the top 25 reasons for physician consultations by patients.

Sexually active women are at increased risk for vaginitis because the presence of semen in the vagina may raise the pH and thereby allow for a proliferation of pathogenic anaerobic bacteria. It is well known that the risk of vaginitis and all STDs is increased in women with multiple sex partners.

This discussion will concentrate on the three conditions that are the most common causes of infective vaginitis—candidiasis, trichomoniasis, and bacterial vaginosis (BV). It should be noted, however, that more obscure infective agents can also cause vulvovaginitis. Some of these include various gram-positive and gramnegative microorganisms, Mobiluncus species, Chlamydia trachomatis, herpes simplex virus, human papillomavirus, Enterobius vermicularis (pinworm), and Giardia lamblia.

In addition to infective causes, allergic or chemically irritative vaginitis can be caused by a great number of substances in our environment. In these situations patients demonstrate long-standing symptoms or recurrent symptoms of vaginal irritation with no demonstrable cause of infection. A detailed history is necessary to disclose the offending substance. The patient should be asked to compile a list of all materials that have come in contact with her perineum. Common materials that exhibit potential irritant effects include deodorants, soaps, vaginal sprays, tampons or pads, colored or perfumed toilet tissue, bubble bath, laundry detergents (especially those with enzymes), fabric softeners, frequent minipad use, tight-fitting synthetic underwear, swimming pools or hot tubs, and vaginal contraceptives or condoms.4

To understand the epidemiology of vaginitis, we must first discuss some basic definitions. Epidemiology is a branch of medical science that deals with the incidence, distribution, and control of disease in a population. It is the sum of factors controlling the presence or absence of a disease or pathology.5

Epidemiology is concerned with patterns of disease occurrence in human populations and the factors that influence these patterns. The epidemiologist is interested in disease as it relates to time, place, and person. To this end, epidemiologists may attempt to determine if a disease's characteristics are changing over time or if it is found in a specific geographic locale. The personal characteristics regarding the demography, biology, socioeconomic factors, individual habits and attributes, and heritable traits associated with disease are also pursued.6

The frequency with which a disease is encountered can be expressed by the terms prevalence and incidence. These two terms are different and are not interchangeable. Prevalence defines the number of cases of a disease process present in the population at risk at a specific time. The populaton at risk can be the entire population of an area but is usually limited to those persons who have been tested for the specific condition. Incidence is defined as the number of new cases that develop in a given population during a defined time period.7,8

Vaginitis is an ancient disease and was described by Hippocrates. In the first century AD, Soranus made the following reference to vaginal discharge: "According to Asclepiades and some others there are two different kinds of flux (for one kind is red, the other is watery and white), whereas according to Demetrius the differences lie in color and action.... In action one kind of flux is inactive and causes neither irritation nor pain, whereas the other kind causes irritation and erosion and brings on a painful sensation at the time of discharge."9

Epidemiology of candidiasis

In this century, the incidence of vaginal candidiasis has increased dramatically. In England the incidence has varied between 28% and 37%. According to Bingham, 10 an infectious disease expert who annually monitors the number of cases of genital yeast infections at STD clinics, between 1971 and 1981 there has been a 42% increase in the incidence of vaginal candidiasis (Fig. 1).

In the United States, mycotic vaginitis has also increased significantly. Between 1980 and 1990, the incidence has nearly doubled. This is associated with an 80% increase in the number of prescriptions written to treat yeast infections in the same time span. It is also significant that the percentage of non-albicans Candida infections is also rising dramatically,1,11

In the 1970s, the incidence of non-albicans vaginal infections was approximately 5% to 10%, and in the 1980s it increased from 15% to 25%. A study in the 1980s involving 728 isolates from women with vaginal candidiasis showed a prevalance of 21.3% nonalbicans species (Table I). In evaluating these data, no specific relationship was discernible between the presence of predisposing factors or the use of previous medication and the occurrence of non-albicans species.

Speciation of these non-albicans species reveals that between 1963 and 1987 (15 studies), the incidence of C. tropicalis increased from 1.3% in the 1970s to 8.2% in the 1980s. C. glabrata also increased over this same

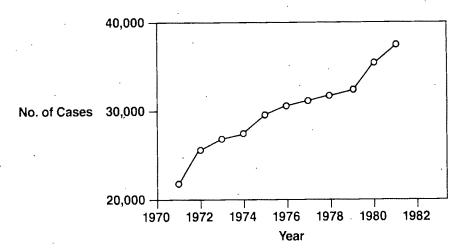


Fig. 1. Evolution of vaginal candidiasis in the United Kingdom; cases collected from STD clinics only.

Table I. Incidence of non-albicans Candida vaginitis in the 1970s and 1980s*

Species	1970s (9 studies)	1980s (7 studies)
C. albicans	1143 (90.1%)	573 (78.7%)
Non-albicans	126 (9.9%)	155 (21.3%)
Total isolates	1269	728

^{*}Survey included only patients with signs and symptoms of vaginitis.

time interval from 4.5% to 6.7% of specific yeast isolates. It is apparent that during these 3 decades, there has been a shift toward increasing percentages of non-albicans infections.

Epidemiology of trichomoniasis

In the United States, Joseph G. Lossick has recently published a report on the epidemiology of vaginal trichomoniasis. In his paper Lossick (personal communication) cites preliminary information on the incidence of *Trichomonas* vaginitis and BV in one STD clinic population.

Vaginitis data were obtained from the National Disease and Therapeutic Index survey, which is composed of a nationally stratified random sample of office-based practice physicians in 19 primary care specialties. More than 2100 physicians report on patient diagnoses and drug use (by individual case report) during a 20-day period each quarter. Physician visit frequency estimates are then extrapolated from these data. In this way crude estimates of the frequency and distribution of diseases seen in the office-based medical setting are developed. These data are particularly relevant for infections ordinarily treated on an outpatient basis, such as trichomoniasis. As with other stratified random sampling schemes, these data are sometimes characterized

by broad confidence intervals or error rates typically associated with population estimates that are developed from a small number of disease occurrences. Based on the size of the visit, volume estimate error rates vary. Statistical procedures have been used to answer questions concering the precision of case definitions and the consistency of the National Disease and Therapeutic Index data over time. The primary purpose was to identify patterns and trends rather than specific disease frequencies.¹²

Patterns of physician visits for *Trichomonas* vaginitis during the 22-year period from 1966 to 1988 show that there has been an approximate 40% decrease during this time span (Fig. 2). These data correlate well with worldwide trends in the occurrence of *Trichomonas*.

Using frequency of physician visits as a reference point, when physician visits are separated into specific geographic regions (West, South, Midwest, and Northeast), one can see differences and similarities in each region. The distribution of infection appears to vary by region, with the highest frequency occurring in the South and the lowest in the West. All areas of the United States have demonstrated similar decreases in morbidity except in the southern states, where the decrease began approximately 5 years later (1980) and has been only half of that seen in other geographic areas (Fig. 3).¹³

Similarly, data on first physician visit demonstrate a higher frequency of visits for black women with *Trichomonas* vaginitis, which is consistent with worldwide data. Fig. 4 shows the estimated U.S. race-specific first visit rate for *Trichomonas* per 100,000 women between 1982 and 1987 (race data were not available before 1982). The data suggest that black women sought treatment for *Trichomonas vaginalis* infections about four times more frequently than did white women. During the study period, first physician visits for

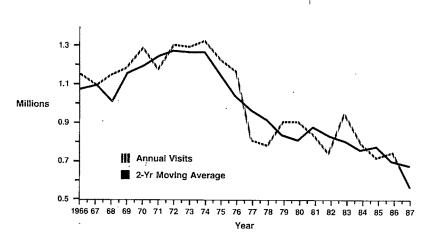


Fig. 2. Physician visits for trichomonal vaginitis, National Disease and Therapeutic Index survey data.

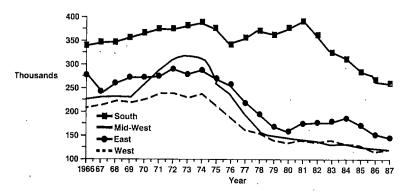


Fig. 3. Physician visits for trichomonal vaginitis by geographic region, United States, National Disease and Therapeutic Index survey data.

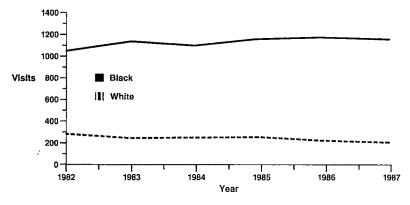


Fig. 4. First trichomonal physician visit rates/100,000, National Disease and Therapeutic Index survey data.

trichomoniasis increased slightly for black women, whereas there was a decrease in visits by white women. Although a higher proportion of black women sought treatment for trichomoniasis, almost two thirds of all physician visits for this infection occurred in white females (Fig. 5). Targeting resources totally to a minority population with a high prevalence may be ineffective, because if all T. vaginalis infections in black females

were eradicated, the largest proportion of infected women would still remain (Fig. 5).12,14

Increasing age and maturity does not decrease a woman's risk of acquiring trichomoniasis; however, the National Disease and Therapeutic Index data show that this infection is predominantly a disease of young women. Fig. 6 shows the age distribution of first physician visits for Trichomonas, and about two thirds of

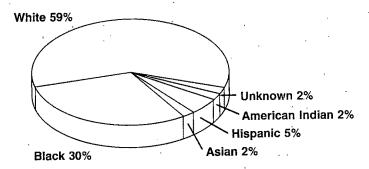


Fig. 5. Racial distribution of first visits for trichomonal vaginitis infections, United States, National Disease and Therapeutic Index survey data.

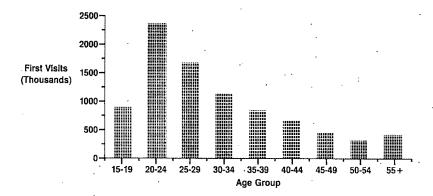


Fig. 6. Age group distribution of first physician visits for trichomonal vaginitis from 1982 to 1987, United States, National Disease and Therapeutic Index survey data.

these visits were made by women under 30 years of age.

Cases of *Trichomonas* vaginitis are distributed widely throughout the continental United States, but there is a higher incidence in the South. Although frequency of first visit is highest in black women and age does not play a role in the patient's susceptibility to *Trichomonas*, most of the infections occur in young white women. However, overall a significant decrease has occurred in the incidence of *Trichomonas* during the past 15 years.

Epidemiology of BV

Lossick developed data on the incidence of BV from patients attending an STD clinic in Columbus, Ohio. The study population consisted of 10,500 women who attended this clinic between 1985 and 1987. BV was diagnosed solely from clinical findings based on established criteria (signs and symptoms, vaginal pH, wet preparation findings, etc.). Because STD clinic populations are not typical for the average female population with venereal disease, these data do not indicate a quantification of risk factors. Therefore epidemiologic characteristics of BV were compared with those of gonorrhea in the same patient population. Total cases include 2000 patients with BV and 2500 with gonorrhea.^{15, 16}

At the STD clinic, frequencies of BV, C. trachomatis, Neisseria gonorrhoeae, and T. vaginalis infections were 17.7%, 25.3%, 24.4%, and 24.9%, respectively. The age distribution of BV was significantly different from that seen in gonorrhea (Fig. 7). In contrast to gonorrhea, which showed an inverse relationship between age and infection rates, the frequencies of BV varied little by age except at the extremes. In sharp contrast to gonorrhea, which was most prevalent in black women, there was no racial prevalence in BV (Fig. 8).

Women with BV were more likely to have had a history of STD: 54% of women with BV reported at least one prior STD versus 36% of women with gonorrhea. Women with gonorrhea came more frequently from inner-city areas, whereas there was no geographic clustering of patients with BV (Fig. 9). Women with gonorrhea were more likely to use oral contraceptives or no contraception, whereas women with BV were more likely to use intrauterine devices, condoms, and diaphragms (Fig. 10).

The descriptive epidemiology of BV in this study differs significantly from that of gonorrhea. Clinical patients with BV were more widely distributed geographically than those with gonorrhea and were neither racially nor age group clustered. Although BV can com-

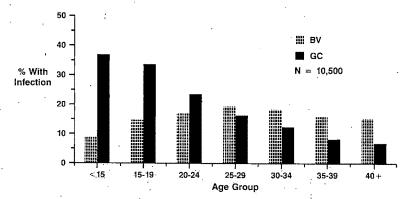


Fig. 7. Age group frequencies of BV and gonorrhea infection. GC, Gonorrhea. (From Lossick JG. Rev Infect Dis 1990;12[suppl 6]:655-81, by permission of The University of Chicago.)

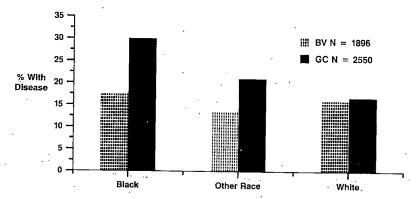


Fig. 8. Racial distribution of BV and gonorrhea cases. GC, Gonorrhea. (From Lossick JG. Rev Infect Dis 1990;12[suppl 6]:665-81, by permission of The University of Chicago.)

monly affect women with gonorrhea, these data indicate that BV affects a much broader spectrum of women than does gonorrhea and in all probability affects women not at high risk for gonorrhea infection.

Epidemiology in Scandinavia

Moi has completed a recent evaluation of the incidence of vaginitis in the Scandinavian countries. As with other countries of the world. vaginitis caused by *Candida, Trichomonas*, or anaerobic organisms is not a reportable disease, and therefore other evaluations must be used to develop some sense of frequency. Prevalence figures are only available from a limited number of surveys compiled from data from STD clinics.¹⁷

In the discussion of trichomoniasis, Moi notes that until 20 to 30 years ago, the occurrence of *T. vaginalis* in specific subpopulations of the Scandinavian countries were correlated with sexual promiscuity. Metronidazole was registered in Sweden in 1957 and within 2 years in the other Scandinavian countries. Since then, the natural course of the disease has been altered through the use of metronidazole, and there has been a steady decline in the incidence of cases.

In contrast to the work of Lossick, Moi states that in

a 1969 study by Bjerre, Papanicolaou smears were evaluated for the presence of *Trichomonas*, and increasing prevalence of *Trichomonas* was found with the increased age of the patient. Teenagers were infected in 3% of cases, whereas menopausal women were infected 18% of the time, with a mean incidence of 9.6%. In other Papanicolaou smear surveys from Sweden and Denmark done during the 1960s, the prevalence of *T. vaginalis* varied from 6.5% to 28%. The highest incidence was found in the port city of Frederiksberg, a part of metropolitan Copenhagen. This high yield was attributed to a more precise evaluation of the Papanicolaou smears and also to a more cosmopolitan population in that area.

In a study from Ostergotland, a positive correlation was reported between the finding of *Trichomonas* in Papanicolaou smears and the occurrence of cervical dysplasia. Thirty percent of women with cancer in situ of the cervix had associated trichomonal infection, whereas only 7.4% of the entire group had *Trichomonas* present. The presence of *Trichomonas* in Copenhagen has decreased from 21% in 1977 to 0.4% in 1988.

In a study from the town of Malmö, Sweden, the number of newly diagnosed cases of trichomoniasis

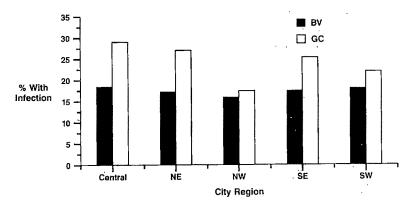


Fig. 9. Distribution of BV and gonorrhea cases by city region. GC, Gonorrhea. (From Lossick JG. Rev Infect Dis 1990;12[suppl 6]:665-81, by permission of The University of Chicago.)

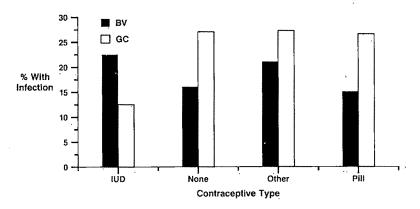


Fig. 10. Contraceptive practices and the frequencies of BV and gonorrhea. GC, Gonorrhea. (From Lossick [G. Rev Infect Dis 1990;12[suppl 6]:665-81, by permission of The University of Chicago.)

from 1975 to 1980 remained at approximately 100 per year. During that same span, the yearly number of cases of gonorrhea decreased from 550 to 300. By 1985 gonorrhea had decreased to 100 cases per year, a prevalence of 19%, whereas trichomoniasis had decreased 5 to 28 cases, a prevalence of 5%. In 1988, 8 cases of trichomoniasis and 18 cases of gonorrhea were diagnosed, a prevalence of 1% and 2%, respectively.¹⁷

In Gothenberg on the west coast of Sweden, there was a 1% to 2% prevalence of trichomoniasis in the STD clinic from 1986 to 1988. Gonorrhea was virtually unseen, with a prevalence of less than 0.5% of new patients.

In Sweden, most causes of trichomoniasis are now seen in women 30 years of age or older. During the past 5 years, about 20% of all new cases of *Trichomonas* were reported as arising during holiday trips to the Mediterranean area.

In a report from the Swedish State Bacteriologic Laboratory in 1989, 1300 cases of gonorrhea were reported in Sweden during calendar year 1988, with a monthly incidence in 1989 of less than 100 cases; trichomoniasis showed a similar marked decline in prevalence. Specific

reasons for the decline in gonorrhea and trichomoniasis in Sweden are believed to hinge on the fact that both these STDs tend to affect the same patients. Prophylactic partner treatment of trichomoniasis without examination has been practiced for more than 20 years in Sweden. Moi¹⁷ postulates that the more frequent use of condoms and decreased promiscuity because of concern about acquired immunodeficiency syndrome are also significant factors in the decrease in *Trichomonas*.

Candidal vulvovaginitis

In the evaluation of women without symptoms in Scandinavia, Moi reports a 15% incidence of candidal carriage. No significant changes in the incidence of vulvovaginal candidiasis have been reported from the Scandinavian countries. In the Oslo STD clinic, vaginal culture for yeast is taken from all women irrespective of symptoms, and for the past 5 years the prevalence has been 28% to 30%. This includes mycotic infections that occur after broad-spectrum antibiotic treatment.

In a study from Stockholm in 1975, the prevalence of *Candida albicans* as determined by culture was 17.3% (233/1347 women) in women attending an STD clinic.

Forty-five of these women had no symptoms of disease. The prevalence of symptoms of candidal vulvovaginitis was 13.4%.

Bacterial vaginosis

In 1986, 1100 of 3500 women attending the STD clinic in Copenhagen had BV, with a prevalence of 30%. The diagnosis was made by clinical criteria with confirmation on wet mount preparations. In Uppsala, Sweden, in 1984, a similar incidence of 33% was reported from the STD clinic in Orebro.

In a Swedish survey of 8000 Papanicolaou smears conducted by Larson,17 the prevalence of BV was 15%. The smears were from women 30 years of age or older attending a cancer screening program in 1976. It was concluded that there was a positive correlation between BV and cervical dysplasia. In both BV and trichomoniasis, foul-smelling amines are produced by the action of anaerobes, and it is believed that some of these amines are carcinogenic cofactors in the production of cervical carcinoma.

In a study of 518 women applying for legal abortion, 170 (33%) had BV.

A recent analysis of women attending an STD clinic in Orebro, Sweden, has been completed (1984 to 1988), in which 3800 new visits were compiled. Seventy percent of the women were 24 years old or younger, and most were serially monogamous with stable spousal relationships. Using established clinical criteria, the mean prevalence of BV was 26%. This prevalence increased significantly with age, with each 5-year group having about a 5% higher prevalence of BV. Twenty percent of the teenagers had BV, whereas more than 40% of the women in the 40- to 45-year-old group had positive findings.17 There was a positive correlation between BV and chlamydial infection, whereas there was a negative correlation between vulvovaginal candidiasis and human papillomavirus infection.

The correlation between BV and various contraceptives was calculated in the younger age group (below age 25 years). There was a significant negative correlation between the use of oral contraceptives and BV. There was also a negative correlation between the use of barrier methods of contraception and BV but a positive correlation with an intrauterine device in situ. Oral contraceptives have a protective effect against Trichomonas, possibly as a result of the acidic Lactobacillus predominant in vaginal flora. This same effect could explain the protective effect that oral contraceptives confer on BV.

Summary

Both trichomoniasis and gonorrhea have shown a marked decrease during the past decade in Scandinavia. The prevalance of trichomoniasis is now reported as less than 1% in STD clinics. The prevalence of vulvovaginal candidiasis in STDs in Scandinavia has remained relatively stable at 10% to 30% during the past 5 years without any obvious change in trend. The prevalence of BV in several STD clinic populations has been reported to vary between 15% and 33%.

It is obvious that no studies can document the actual specific incidences of vaginitis because vaginitis is not a reportable disease, and attempts to arrive at a precise figure can only be approximated by peripheral evaluations.

Studies that involve registrants of STD clinic populations are obviously flawed, because only a small spectrum of the entire female population attends these clinics, and those who do are more likely to be carriers of vaginal pathogens.

Studies on the Scandinavian population have been well done because of the meticulous record-keeping in the state-controlled medical system, but the incidence of disease is that of a homogenous temperate climate population. Data suggest that the incidence of vaginitis is greater in the more humid subtropical countries, where both infection and transmission of the disease vectors are facilitated.

Studies on the incidence of vaginitis in the United States are based on a very small sampling of patients but indicate a higher incidence of vaginitis in the southern states compared with those in the North and the West.

From a worldwide perspective, it is evident that the number of cases of trichomoniasis has decreased during the past 15 to 20 years, largely as a result of better diagnosis and the use of metronidazole as a systemic medication. Although there has been documentation of metronidazole-resistant trichomonads since 1963, it seems that resistance is not all or none and that by increasing the time exposure and dosage of metronidazole, the vast majority of trichomonads can be eradicated. With several new anti-Trichomonas drugs in clinical trials, it seems that Trichomonas will continue on its downward spiral.

In contrast, the number of new cases of candidal vaginitis has been on the increase during the past decade, and this problem has been compounded by the appearance of an increasing percentage of non-albicans species. This has led to the development of candidal vulvovaginitis that is more resistant to the usual drugs of choice. This situation will require more elaborate strategies in the development of more efficient drugs and the manipulation of the immune response to bring about a decrease in the number of cases of candidal vaginitis.

BV continues to be the leading variety of vaginal infection in the United States. With an increased understanding of the diverse flora in the vagina and the interplay of many species in the production of this disease entity, it is hoped that this problem will soon be more readily treated and controlled.

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Vulvitis and vulvovaginitis: Cutaneous considerations

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Vulvar dermatoses (previously dystrophies) include psoriasis, allergic or irritant reactions, lichen sclerosus, lichen simplex chronicus, lichen planus, and tinea. Some of these have bullous or erosive forms, but they differ from the immune-mediated vesiculobullous disease group, which includes vulvar pemphigus, benign familial pemphigus, pemphigoid, linear IgA disease, and dermatitis herpetiformis. Vulvar ulcers can occur in dermatoses resulting from systemic disease (Behçet's syndrome, lupus, pellagra, and Reiter's disease) or malignancies resembling dermatoses (extramammary Paget's disease, squamous cell carcinoma, and vulvar intraepithelial neoplasia). Many vulvar dermatoses itch or burn. Vulvodynia occurs with irritant and allergic dermatitis, vulvar dermatoses, complications of steroid use, candidiasis, papillomatosis, vestibulitis, or essential (dysesthetic) vulvodynia. Diagnostic tests (potassium hydroxide, cultures, and biopsy) should establish the diagnosis and therapy should be specific. Few skin diseases are curable but all are treatable; effective management is defined by whether a medication reliably controls outbreaks or symptoms when it is used. Patience is recommended, because treatment may take weeks or months. (AM J OBSTET GYNECOL 1991;165:1176-82.)

Key words: Vulvar dermatoses, vulvodynia, psoriasis, lichen sclerosus, lichen simplex chronicus, lichen planus, tinea, vulvar ulcers

Vulvar itching and burning are not always symptoms of vaginitis; they are also typical of dermatitis or primary skin diseases. Patients who believe they have vaginitis often self-treat with medications that can complicate subsequent correct diagnosis and therapy. This article reviews the spectrum and differential diagnosis of vulvar cutaneous disease and symptoms (pruritus vulvae and vulvodynia). Principles of diagnosis and therapy include finer points and pitfalls of dermatologic management.

The term "dystrophy," which was once part of the

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International Society for the Study of Vulvar Disease classification scheme for cutaneous vulvar disorders (atrophic, hyperplastic, and mixed), has now been replaced by "dermatoses." The new categories are as follows: (1) lichen sclerosus, (2) other dermatoses, and (3) squamous cell hyperplasia (formerly hyperplastic dystrophy or nonspecific epidermal proliferation). The second group includes dermatologic disorders previously classified as dystrophies: psoriasis, allergic or irritant reactions, lichen simplex chronicus, and lichen planus. Because of shared morphologic characteristics, dermatologists call these disorders the papulosquamous dermatoses and include tinea and disorders also found on other areas of the body besides the

Another broad dermatologic category is the vesiculobullous diseases, which have blisters and secondary erosions or ulcers. Those with vulvar or vulvovaginal involvement will be discussed, as will ulcerative dermatoses resulting from systemic disease. Symptoms of vulvar disorders unrelated to vaginitis also include vulvar vestibulitis, vestibular papillomatosis, and essential (dysesthetic) vulvodynia.

Dermatologic diagnosis

Pruritus vulvae combines the subjective complaint of itching with the objective finding of leathery plaques (lichenification); visible scratch marks (excoriations) also confirm the diagnosis (Fig. 1, p. 1223). Burning is a "hands-off" symptom, and patients who do not itch may have minimal skin changes. The International Society for the Study of Vulvar Diseases defines vulvodynia as chronic vulvar discomfort, especially that characterized by the patient's complaint of burning, stinging, irritation, or rawness.2 Vulvodynia, a long-term rather than a short-term problem, has been linked in some cases to vulvar dermatoses, recurrent vaginitis (especially candidiasis), human papillomavirus, and dysesthesias.3

Contact and irritant dermatitis

Understanding the difference between irritant and allergic reactions helps in the choice of appropriate medications for the patient who claims she is "allergic to everything." Irritant reactions are common; true allergies are rare. The report of immediate burning or stinging when a medicine is applied to the vulva is typical of an irritant reaction; itching is unusual. Erythema and sometimes edema last for minutes to hours; vesicles do not form, and the affected skin may look dry or parched. Common irritants include soaps, medications (lidocaine and crotamiton), or ingredients such as alcohol and propylene glycol. Irritated, dermatitic skin can be soothed with sitz baths, Burow's compresses, and bland emollients (diaper ointment and vegetable shortening) for a few days. If the offending agent does not sting normal skin on other areas of the body, it can

often be applied to the vulva when initial reactions subside.

An allergic reaction signifies cell-mediated immunity. A familiar example is poison ivy, which causes itchy blisters on the skin of persons sensitized to the plant by prior exposure. The rash takes 48 hours to develop, affects skin wherever the allergen touched, and lasts for about 3 weeks. Treatment with cool compresses and potent topical steroid creams may help, but systemic steroids (30 to 60 mg of prednisone daily) will control severe outbreaks. Familiar medications that cause allergic reactions include topical diphenhydramine, neomycin, and ethylenediamine, which is a preservative in a popular nystatin-neomycin-triamicinolone combination cream.

Chronic dermatitis and lichen simplex chronicus

Chronic contact or irritant reactions itch, as do tinea or Candida infections; itching for any reason generates a nonspecific scratch response (Fig. 2, p. 1223). If scratching continues long enough, the result is lichen simplex chronicus, which is the objective finding in the subjective complaint of pruritus. Lichenification describes classic changes in the skin: thickened and leathery with enhanced skin-surface markings. Measures to , help the patient overcome the itch-scratch cycle include topical steroids to decrease inflammation and gradually flatten the thick plaques of lichen simplex chronicus and antihistamines for a soporific effect. Potent steroids (desoximetasone or fluocinonide) may be used for the first 4 to 6 weeks to control the inflammatory response, followed by a tapered dose of lower potency. Triamcinolone acetonide 0.1% may be used once or twice daily for as long as 2 months as lesions improve. (When lichen simplex chronicus lesions are dermatitic or "wet," a course of oral erythromycin or dicloxacillin speeds recovery.) Hydrocortisone 1% is excellent maintenance therapy for use "as needed." Localized thick plaques of lichen simplex chronicus on the vulva respond well to intralesional injection of triamcinolone acetonide suspension, 5 to 10 mg/ml. Side effects include epidermal and dermal atrophy and depigmentation, but dermatologists still prefer intralesional steroids over alcohol injection, which can cause tissue slough and scarring.

Side effects of topical steroids

The use of potent topical steroids should be limited to brief use for short-term problems; this avoids the many complications associated with long-term use. The best-known consequences include telangiectasias, easy bruisability, skin fragility, and striae formation. Striae are nonreversible and are particularly common in intertriginous areas, such as the upper thigh. The use of potent steroids on the vulva can cause "periorificial dermatitis," a rebound inflammatory reaction with erythema and a burning sensation that flares as the steroid

Table I. Differential diagnosis of the vulvar dermatoses

Condition	Clinical appearance	Diagnostic test	Therapy
Psoriasis	Red plaques with silvery scale; also on knees, elbows, scalp; nail pitting	Clinical appearance; cutaneous biopsy	Topical steroids (triamcinolone 0.1%); systemic antimetabolitics Rx if severe
Seborrheic derma- titis	Scaling/erythema; also on eyebrows, nasola- bial folds, hairline, occ axillae	Clinical appearance; KOH preparation of scale negative	Dandruff shampoos, hydrocortisone 1% cream
Dermatophyte (tinea cruris)	Annular plaque with central clearing and peripheral scale; KOH—>hyphae	KOH preparation of scale positive	Topical imidazole creams bid until clear for 1 wk
Chronic dermatitis (contact or irritant)	Often eczematous and oozing; may involve congruent areas, eye- lids; may generalize	Careful history and patch testing if indicated	Cool compresses, Crisco or hydrocortisone 2.5% ointment, no allergens
Lichen simplex chronicus	Thick, furrowed vulva; other common sites are ankle, arm, or nape of neck	Cutaneous biopsy	Triamcinolone 0.1% ointment for 4-6 wk; rule out vaginal <i>Candida</i>
Lichen planus	"Purple polygonal pap- ules and plaques," lacy white pattern or erosions on oral and vulvar mucosa; wrists, shins common	Cutaneous biopsy	Topical steroids: cream or suppositories
Lichen sclerosus	White, wrinkly; usually only vulva, anus ("keyhole" pattern); dermis thick, epidermis atrophic; occ patchy on trunk	Cutaneous biopsy	Topical testosterone propionate 2% in petrolatum or Aquaphor bid; topi- cal progesterone in children

KOH, Potassium hydroxide; bid, twice daily.

is withdrawn (Fig. 3, p. 1223). A cycle of symptomatic vulvar dermatitis can be established as the patient treats the erythema and discomfort with the same potent topical steroids that started the problem. A combination cream of clotrimazole (antifungal) and betamethasone dipropionate (topical steroid) has been a frequent offender in the development of periorificial dermatitis, and it should not be used for chronic vulvar dermatoses.

Dermatoses

Most vulvar eruptions are "papulosquamous" (thick and scaly) dermatoses, and they are the most common skin diseases in the general population. Erosions or even ulcers may result from excessive scratching, but these are secondary to excoriation and do not change the categorization of the disease process. If the patient denies scratching and describes blisters or primary ulcers, she may have a vesiculobullous disease. The differential diagnosis of red, scaly, cutaneous plaques includes psoriasis, seborrheic dermatitis, lichen simplex chronicus, lichen planus, lichen sclerosus, and dermatophyte (tinea) infections (Table I).

Psoriasis. The most common (vulgaris) pattern of psoriasis on the body is red plaques with thick silverywhite scales on the scalp, knees, elbows, and sacrum.

On the vulva, the appearance varies from moist greyish plaques on the labia majora to glossy red plaques without scale in skin folds. Topical hydrocortisone is safe for prolonged use, even in pregnancy, and genital lesions usually respond well. For painful fissuring and maceration, short-term intermittent (no more than 2 to 3 weeks) use of fluorinated steroid creams may reduce inflammation and encourage healing.

Tinea. Chronic tinea cruris is uncommon in women; tinea versicolor is more frequent than classic dermatophyte infection. Tinea lesions can take many forms; thus any scaly eruption on the vulva should be scraped for a potassium hydroxide examination. Topical antifungals are effective in all but the most widespread eruptions or deep follicular infections. The imidazole compounds (clotrimazole and miconazole) are excellent; nystatin and terconazole, a triazole, are indicated only for *Candida* infections. Twice-daily treatment is best (rub in well); it should continue for at least a week after lesions clear. For chronic tinea infections, systemic treatment with griseofulvin or ketoconazole should be considered; a 4- to 6-week course is effective in patients who are nonimmunosuppressed.

Lichen planus (erosive vaginitis). This papulosquamous disorder has two distinctly different expressions: smooth shiny pruritic flat-topped papules on the skin and white patches or erosions on mucous membranes. Lichen planus papules are characteristic and histologically distinctive, but mucous membrane erosions are nonspecific. Most patients have both cutaneous and mucosal lesions, but either type may appear in a given patient. Lichen planus is probably the major cause of chronic erosive vaginitis (Fig. 4, p. 1224).

The classic definition of lichen planus includes "five Ps": purplish, pruritic, polygonal papules and plaques. The polygonal outline is subtle, suggesting that the borders of small plaques tend to be defined by the crosshatch pattern of minor skin lines. Scaling is faint and more of a whitish-gray surface streaking (Wickham's striae), a lacy pattern often seen on the buccal mucosa. Lesions may line up along scars or scratch marks (the Koebner phenomenon, seen also in psoriasis). Additional lesions may appear on the volar wrist, lumbar back, medial thighs, ankles, and shins.

Topical steroids are the treatment of choice for lichen planus. High-potency preparations may be necessary, and intralesional triamcinolone is probably the best treatment for localized hypertrophic lichen planus. Erosive vaginitis may be controlled initially with potent topical steroid creams (clobetasol propionate, or desoximetasone) inserted vaginally each night for 1 or 2 weeks, with gradual tapering of the dosage, switching to hydrocortisone suppositories or creams as improvement occurs. Oral etretinate (Tegison) helps severe mucosal lichen planus, but teratogenicity and a half-life of many months contraindicate its use in women who are pregnant, who plan to become pregnant, or who may become pregnant during therapy.

Lichen sclerosus. Vulvar lichen sclerosus is a major genital dermatosis familiar to gynecologists as a symmetric "keyhole" pattern on the vulva. The sclerotic dermis is white with a thin atrophic epidermis that may be finely wrinkled or scaly (Fig. 5, p. 1224). Bruises, lacerations, and purpura are common on this fragile skin, especially when pruritus is present; blisters or ulcers may even develop in some lesions. The architecture of the vulva disappears as the labia minora resorb and efface; this finding may sometimes precede extensive vulvar whitening and is an early diagnostic sign. Symptoms of itching or burning are variable; they do not necessarily correlate with the size or severity of lesions.

Childhood lichen sclerosus is not uncommon; perianal or vulvar skin is typically pale and wrinkled on the surface, and petechiae and purpura make child abuse a possible diagnostic consideration. Vulvar lesions improve by puberty, but few clear completely. Hydrocortisone 1% cream or ointment alternated with bland emollients is frequently the only treatment necessary to control childhood lichen sclerosus; testosterone ointment should not be used.

Lichen sclerosus is the only vulvar dermatosis that sometimes responds to topical testosterone therapy (2%

testosterone propionate in petrolatum, formulated by the pharmacy). Prepubertal girls should be given progesterone ointment instead (100 mg of progesterone in oil mixed with I ounce of petrolatum). A treatment trial should be undertaken for at least 2 months of twice-daily application, which may be tapered to a maintenance dosage if a therapeutic effect is achieved. Many patients have no resolution of lesions; continued use of testosterone merely slows the progression of the disease and keeps involved skin from tearing. It is possible for symptoms to persist even while the skin's appearance improves, and topical steroids may be added temporarily for symptomatic relief of itching. Excisional surgery is not curative, because lesions can recur in grafted skin. However, introital narrowing can be surgically corrected, and topical therapy should be continued as soon as healing is complete.

Vesiculobullous diseases

Vesicles and bullae are the typical lesions for this group of dermatologic disorders, which can also be erosive on mucous membranes or the vulva. Differentiation between bullous diseases requires a biopsy for histopathologic examination and direct immunofluorescence, which requires a specific tissue fixative (not formalin). Other dermatologic disorders that may be bullous or erosive include acute contact dermatitis, lichen planus, and lichen sclerosus (Table II).

Erythema multiforme. Recurrent painful oral and genital ulcers are typical of erythema multiforme, which is usually associated with lesions elsewhere on the body, especially the palms and soles ("target" lesions). The most common association with recurrent erythema multiforme is a preceding flare of herpes simplex, but drug allergies are also a consideration. This condition may be very difficult to differentiate from Behçet's syndrome and aphthosis, both of which have episodic oral and genital ulceration.

Pemphigus. Pemphigus is an immune-mediated skin disorder in which there is destruction of the intercellular cement substance between epidermal cells. The result is fragmentation of the top layer of the skin with formation of blisters and erosions. Biopsy for immunofluorescence establishes the diagnosis, and high-dose systemic steroids (prednisone, up to 300 mg daily for widespread pemphigus vulgaris) may be required to bring about initial control. Immunosuppressive drugs are used for maintenance therapy.

Benign familial pemphigus. Benign familial pemphigus (Hailey-Hailey disease) is a rare autosomal dominant dermatosis; 70% of patients have a positive family history. A chronic, recurrent, erosive papular eruption in the groin (and other skin folds) persists despite a variety of palliative treatment regimens. Disease severity varies from patient to patient but typically waxes and wanes. Treatment is topical steroids and antibiotics

Table II. Differential diagnoses of vulvar erosions and blisters

Erosions or blisters	Diagnosis	Course	Patient age	Scarring	Oral	Body lesions
Dermatoses				•		
Erythema multi- forme	Histopathology	Recurs (HSV)	Any age	No	Yes	Palms and soles
Pemphigus	Immunofluorescence	Chronic	>Middle age	No	Yes	Yes
Benign familial pemphigus	Histopathology	Chronic	Postpuberty	No ·	No	Intertriginous
Bullous pemphigoid	Immunofluorescence	Chronic	Elderly	No .	No	Various ,
Linear IgA disease	Immunofluorescence	Chronic	Childhood `	No	No	Various
Lichen planus	Histopathology	Chronic	Postpuberty	Yes	Yes	Wrists, shins
Lichen sclerosus	Histopathology	Chronic	Any age	Yes	No	Rare
Infections						
Ímpetigo	Culture	Episodic	Any age	No ,	No	No
Herpes simplex virus	Culture	Recurrent	Any age	Sometimes	No	No
Herpes zoster	Culture	Episodic	More common With age, HIV	Frequent	No ,	Dermatomal
Systemic diseases					•	
Behçet's	Histopathology	Recurrent	Postpuberty	Common	Yes	Eyes, arthritis
Pellagra .	Niacin deficiency	Episodic	Any age	No	Glossitis	Intertriginous
Lupus erythematosus	Serum tests	Recurrent	Any age	No	Yes	Various
Reiter's	Histopathology	Chronic	>Middle age	No	Ņо	Palms and soles

for secondary infection. Immunosuppressant agents are of little value.

Bullous and cicatricial pemphigoid. More common in elderly persons, bullous pemphigoid lesions are the result of immune deposition at the dermal-epidermal junction at the basement membrane zone. Because there is an almost intact layer of epidermis over the blister roof, bullae are tense and last for days. Cicatricial pemphigoid (benign mucous membrane pemphigoid) typically localizes to certain areas, and the scalp or mucosae are common sites. Scarring is a dominant feature. Histopathologic studies and immunofluorescence should be performed. Control of bullous pemphigoid requires systemic steroids; alternate-day doses are usually less than 80 mg of prednisone.

Dermatitis herpetiformis. The confusing name of dermatitis herpetiformis reflects the appearance of grouped vesicles that may develop anywhere on the body. Immunofluorescence shows aggregates of immunoglobulin A (IgA) in the papillary tips of the dermis in biopsy specimens from anywhere on the skin. An often subclinical gluten-sensitive enteropathy is associated with dermatitis herpetiformis. Treatment is with dapsone or a gluten-free diet.

Pediatric vulvar disease

The need to recognize and establish an early diagnosis of abuse in cases of traumatic genital lesions in children is clearly a priority in preventing further damage. It is also important to recognize that purpura, erosions, and scarring can also be caused by genital cutaneous disease (Table II).

Lichen sclerosus. Lichen sclerosus has been reported as early as infancy and occurs often enough in pre-

pubescent girls that it should be a routine diagnostic consideration. Lichen sclerosus typically produces white scarring around the vagina, anus, or both, and lesions are easily traumatized. Itching lesions of lichen sclerosus may show purpura or lacerations from the patient's scratching, and pain on defectation or urination is a common complaint (for therapy, see above discussion).

Linear IgA disease. Linear IgA disease is named for the immunofluorescence deposition pattern of IgA on microscopic examination. Formerly known as bullous disease of childhood, blisters of this disease may resemble traumatic burns on the patient's skin, and clustered genital lesions suggest herpes simplex. Biopsy samples for histopathologic examination and immunofluorescence are diagnostic.

Bullous impetigo. Secondary infection of the genitalia with *Staphylococcus* or *Streptococcus* is common in pruritic dermatoses such as atopy. Lesions will be oozy and wet, sometimes with pustules. When secondary impetiginization occurs, the patient should be checked for an underlying dermatitis that must also be treated to prevent reinfection. Therapy includes topical measures (wet compresses and hydrocortisone cream) and systemic antibiotics (erythromycin and dicloxacillin).

Vulvar erosions secondary to systemic disease

In some cases, systemic diseases are associated with skin lesions that may involve the genitalia. These vulvar dermatoses are more likely to be encountered on a consultation service than in a general clinic (Table II).

Behçet's syndrome. Oral and genital ulcers alone do not establish the diagnosis or justify therapy for Behçet's syndrome. The diagnosis must be based on a

combination of three or more of the following clinical problems (including oral and genital ulcers): uveitis, cutaneous vasculitis, arthritis, meningoencephalitis, and cutaneous hyperreactivity to minor trauma (pathergy). Behçet's syndrome is often overdiagnosed; aphthosis or herpes simplex are more common causes of recurrent oral and genital ulcers.

Lupus erythematosus. About one fourth of patients with lupus erythematosus will have mucosal lesions, which are usually noticed during disease flares. Treatment of the lesions is symptomatic; systemic therapy is used to control the underlying condition. Recognition that the mucosal erosions in patients with lupus erythematosus may not be herpes simplex can spare her much distress.

Pellagra. Patients with poor nutrition may have dry scaly skin and erythema of mucous membranes, which is the result of multiple vitamin deficiencies and lack of essential fatty acids. The classic tetrad of pellagra (diarrhea, dermatitis, dementia, and death) results from niacin deficiency. Skin signs of pellagra include hyperpigmentation and peeling of intertriginous areas and erosive mucous membranes. The condition responds to a balanced diet.

Reiter's disease. Reiter's disease is a consideration in the differential diagnosis of chronic genital psoriasiform lesions. Mucocutaneous lesions may be pustular or erosive (keratoderma blennorrhagicum); they occur with recurrent symptoms of urethritis, inflammatory eye disease, diarrhea, and inflammatory arthritis. Reiter's disease is associated with HLA B-27.

Cancer

Extramammary Paget's disease. Extramammary Paget's disease is a distinct histopathologic entity that usually appears as a red, velvety, well-demarcated lesion with an eczematoid scaly surface. Because it resembles a benign nonspecific dermatitis, Paget's disease has typically been present for months to years at the time of diagnosis. The average age of patients with Paget's disease is 65 years; thus in this age group it is important to take biopsy specimens of lesions that are not responding to therapy, especially if they are asymptomatic. Excision is the treatment of choice for Paget's disease, with careful systemic evaluation to rule out underlying or associated adenocarcinoma.

Squamous cell carcinoma in lichen sclerosus. Lesions of lichen sclerosus may be the site of vulvar carcinoma in situ in older patients, probably because of chronic inflammation. Ulcerations developing in longstanding lesions of lichen sclerosus should be biopsied at different sites on the lesion border rather than at the base of the ulcer. Elderly patients are often reluctant to return for procedures; thus it is probably best to take biopsy specimens of suspicious lesions as soon as they are discovered.

Vulvar intraepithelial neoplasia (VIN). Condylomata acuminata are usually not difficult to recognize, but long-standing sessile lesions thought to be human papillomavirus (HPV) may be squamous cell carcinoma in situ or VIN, which is often a multifocal disease. VIN lesions may be condylomatous, eczematous, or ulcerated; coloration may vary from white to red or brown. Many patients with VIN mention only pruritus or a rough spot on the skin. Representative lesions should be biopsied to establish a diagnosis before destructive therapy is performed. It should be remembered that patients with multifocal disease can also have dermatitis. A biopsy specimen may show benign disease rather than malignancy.

Vulvodynia

As previously mentioned, vulvodynia is chronic vulvar discomfort with patients complaining of burning, stinging, irritation, or rawness. Candida is only one diagnosis associated with vulvodynia; vulvar dermatoses, papillomatosis, vulvar vestibulitis, and dysesthesias, such as pudendal neuralgia, must also be considered.

Vestibulitis. In the vulvar vestibule are the openings of the urethra and Skene's and Bartholin's glands. Close examination may also reveal the tiny pit-like openings of the minor vestibular glands, which scatter throughout the vestibule and concentrate in the posterior fourchette and in the groove at the base of the hymenal ring. Characteristics of vestibulitis are the patient's complaint of entry dyspareunia, discomfort at the opening of the vagina, and erythema and point tenderness discovered on palpation of the gland orifice with a cotton-tipped applicator.5

The significance of painful red vestibular glands is not entirely clear. Many women who have no problems with intercouse will mention pain on palpation of these glands during a routine examination. Inflammatory vulvovaginitis causes acute discomfort of vestibular gland openings, but this tenderness generally resolves with or soon after treatment of the infection. Chronic vestibulitis, however, persists for months or years, and patients experience not only entry dyspareunia but often have pain when they try to insert tampons. There must be factors other than the vestibular glands that affect this symptom complex, but more detailed study is needed.

Papillomatosis. Vestibular papillae are tiny fibrillary growths in the vestibule that are usually most numerous at the posterior introitus. They are a normal anatomic variant.4 Vestibular papillomatosis describes the presence of multiple papillae that may cover the entire mucosal surface of the labia minora. Papillomatosis can occur with human papillomavirus infection, but HPV is not proved in all cases. Normal women who have no clinical symptoms may have similar findings. Acetowhitening (application of vinegar or 3% to 5% acetic 1182 McKay
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acid solution to the epithelium for 1 to 2 minutes) improves visualization of papillomatosis and parakeratotic changes. In the vulvar vestibule, papillomatosis and acetowhitening are both nonspecific findings of uncertain significance.⁵ In patients with symptoms of vestibular papillomatosis, expert colposcopic examination and acetowhitening should be used primarily for directing biopsies; histopathologic studies should be the basis for therapeutic decisions.

Essential vulvodynia. Essential (dysesthetic) vulvodynia typically occurs in elderly or postmenopausal patients who complain of diffuse and unremitting genital burning without evidence of vestibulitis or cutaneous changes. These patients describe pain patterns similar to postherapeutic neuralgia (postzoster neuralgia) and "glossodynia" (burning tongue), which suggests a problem with cutaneous perception either centrally or at the nerve root. A tricyclic antidepressant (amitriptyline) frequently brings relief at doses of 30 to 50 mg daily.

Summary

The multifactorial nature of the symptoms and physical expression of disease on the vulva complicates the evaluation and management of genital dermatoses. However, a few simple rules can help the clinician avoid the most common errors in diagnosis and treatment.

Because chronic itching usually leads to the development of lichen simplex chronicus, this diagnosis should not be assumed to occur as an isolated finding. One should always look for more than one potential cause for the complaint of itching or burning. Diagnostic tests, such as potassium hydroxide examination and cultures, should be performed routinely, and biopsy studies should be performed on any lesion for which the diagnosis is unclear. A diagnosis must always be made on objective grounds before a course of therapy is initiated. If appropriate therapy for a given diagnosis does not prove to be effective, it is best to reconsider the diagnosis before simply changing the treatment.

Understanding the chronic nature of skin disease is critical; management and not cure is usually the treatment goal. Few recurring skin diseases are curable but all are treatable; patients can be spared frustration when they receive the proper medication early in the course of the disease. The true definition of effective treatment for a dermatosis is whether routine application of a particular medication can control outbreaks or symptoms. Recurrence of a skin problem when medication is discontinued is not a treatment failure, but a sign that therapy has been appropriate and should be continued.

Topical therapy takes time to produce results; medications must be formulated carefully to penetrate a thick scaly skin barrier. It may be necessary to apply topical agents twice daily for as long as 1 week before symptoms begin to abate, and clearing of chronic lesions may take many weeks or months.

"Shotgun" therapy (use of different medications simultaneously or changing them after a few days) without a clear diagnosis is not a good management plan. Not only can medications interfere with one another, but the patient is discouraged by the expense and the feeling that her condition cannot be treated. She may become resentful and think that her treatment is experimental. The best maintenance therapy is often continuous use of bland emollients or mild topical steroids and reassurance that no infection or malignancy has been discovered. Fortunately, "psychosomatic disease" is a less common diagnosis than it was before objective findings in vulvodynia and dyspareunia were described. Careful examination of patients and selection of specific treatment plans can provide relief to many patients who had little hope of effective therapy in the past.

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Vulvovaginal human papillomavirus infections: Clinical implications and management

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The past 2 decades have witnessed an alarming increase in the incidence of human papillomavirus infections. Clinically evident cases represent only a small portion of the infected population, because millions of people have subclinical or latent infection. Human papillomavirus infection is recognized as a precursor to malignancy. Thus it is important to treat clinically evident infection. Treatment is complicated by the ability of the virus to establish latent infection and the lack of an effective antiviral agent. At present treatment is limited to the destruction of obvious and intraepithelial lesions. (AM J OBSTET GYNECOL 1991;165:1183-8.)

Key words: Human papillomavirus infection, condyloma acuminata, laser therapy, α -interferon

During the past 2 decades there has been an alarming increase in the number of patients seen with human papillomavirus (HPV) lesions. Part of the increase is a heightened awareness of the several manifestations of HPV growth; however, the number of cases of condyloma acuminatum has increased 10-fold during the past 15 years, and it is now the most commonly diagnosed sexually transmitted disease in the United States. Moreover, the number of patients with clinically overt disease is just the tip of the iceberg that includes virtually millions of sexually active adults with subclinical or latent infections. DeVillers et al. reported that 10% of more than 9000 West German women with normal Papanicolaou smears tested positive for HPV 16 by filter in situ hybridization. Approximately 30% of these women had a sustained positive test, whereas the others had a subsequent negative test. Some patients had a negative test result on the first screening but had positive findings on a subsequent screening. This suggested that HPV exposure occurs intermittently and that HPV may be a commensal, much like Candida albicans or Staphylococcus aureus. Other countries have reported similar incidences of asymptomatic HPV. Kjaer et al.² reported as high as a 13% rate in Greenland and Denmark. Toon et al.3 reported an 11.5% rate in the United Kingdom. In the United States, Becker et al.4 reported an 8% rate among women attending a university student health service and a 25% rate among women attending a sexually transmitted disease clinic.

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Management

To treat HPV infection of the anogenital tract successfully, the physician must have a basic knowledge of the anatomy of the skin on which the virus is growing. Warts located on the keratinized, hair-bearing areas of the skin tend to produce thick keratin growths. These growths are more resistant to topical chemodestructive therapy than are mucosal lesions, which are softer and more vascular and therefore allow better penetration of the topical agents and greater efficacy. The pilosebaceous appendages of the hair-bearing areas may be infected with HPV and allow regrowth of the wart from the hair shafts and sweat glands. Fortunately, infection does not usually penetrate deeply into these appendages, and destruction directed to the surface is often successful. The physician must also recognize that latent virus is present in normal-appearing skin around the obvious lesion. Regrowth from this latent virus requires weekly reevaluation and treatment until the host has been able to effect its immune response and further expression of the virus is halted. For this reason, the simplest forms of treatment are the best when the patient is in this productive phase of HPV infection.

The simplest treatment is either bichloroacetic acid or trichloroacetic acid. A 50% to 85% solution is used; the water content of the tissues treated neutralizes these dessicant acids. Thus keratinized lesions, which contain little water, are more resistant to the acids. Mucosal lesions are the most suitable warts for treatment. Because the acids contain no toxic agents to absorb, they may be used in the vagina and on the cervix. They are also safe for use during pregnancy.

Laser treatment

For acid failures, the CO₂ laser has gained widespread popularity in the treatment of HPV. When cou-

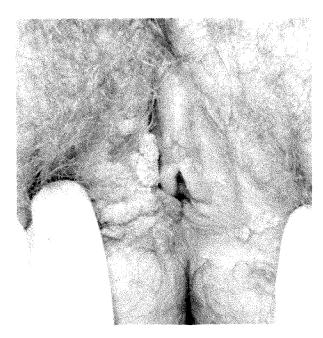


Fig. 1. A 20-year-old woman with recurrent condyloma after multiple treatments including laser treatment.

pled with the operating microscope (colposcope), it allows the surgeon to identify and destroy both microscopic and macroscopic HPV. By learning to identify the dermatologic surgical planes, the surgeon can avoid damage to the underlying dermal architecture and thus avoid scarring.

Tissue destruction from the laser occurs from both immediate vaporization and delayed tissue necrosis. The former can be controlled by observation of the surgical planes; the latter can be controlled by keeping the power density above 750 W/cm³. Power densities below this level lead to excessive tissue necrosis.

Laser vaporization should not be used as initial therapy for patients who are still in the proliferative phase of rapid epithelial and papillary growth formation. It is best used with patients who have developed resistant, thick keratotic lesions unresponsive to local chemical therapy. When used in this manner, the remission rate is approximately 85%. When possible, outpatient treatment with local anesthetic is preferred. The intradermal injection of local anesthetic helps produce a water barrier at the dermoepidermal junction and aids in identifying the dermatologic planes.

The wart produces a thickening of the normal epidermis. The goal is to vaporize this thick epidermal outgrowth without damage to the papillary dermis below. A high-power density should be used, with frequent removal of charred debris by wiping with a wet gauze. The dermoepidermal junction can be recognized by an intact, shining surface with microscopic papillary dermis. Normal-appearing skin around the

wart for a distance of 1 cm should be "flashed." This step is accomplished by rapid movement of the laser beam, which produces a typical crackling sound. The surface of the skin blanches and bubbles appear underneath. This layer is easily wiped away, and the papillary dermis can be observed. Cooling the vulvar skin with cold, moist gauze aids in limiting thermal injury. After surgery the patient's laser wound is cooled for 24 hours and is then treated with topical silver sulfadiazine. Healing takes place in 10 to 14 days without scarring.

The wart that involves skin appendages, such as hair follicles or sebaceous glands, becomes more difficult to treat. The skin appendages penetrate the papillary and reticular dermis, which necessitates a third-degree burn to destroy the entire lesion to that depth. This burn takes several weeks to heal and results in scar formation. Small lesions (<5 mm) can be treated to this depth without significant cicatrization. Larger lesions, however, should not be treated this deeply. Because HPV infection generally involves the superficial portion of the skin appendages, lesions in these patients can be vaporized to the second surgical plane and still preserve the reticular dermis, leaving a foundation over which reepithelialization can take place quickly from the hair follicles.⁵ This plane is recognized by the roughened, yellow base visible through the microscope. Although the entire hair shaft or sebaceous gland is not destroyed, the success rate is satisfactory. Interferon therapy after laser treatment further improves the success rate (Figs. 1 through 4).6 Treatment of warts through the third surgical plane may cause cicatrization and is not advised.

α-Interferon

In 1957 Isaccs and Lindermann⁷ first described interferons as endogenously produced cytokines that protect cells against viral infection. The three major types of interferon are α , β , and γ . α -Interferon is the agent currently used to treat condylomata acuminata. Since 1980, recombinant deoxyribonucleic acid technology has been used to mass produce α -interferon, which has facilitated the completion of clinical trials. Vance et al.8 reported the first randomized controlled trial of interferon. Patients had a single wart injection three times a week for 3 weeks with either 106 U, 105 U, or a placebo. They were then observed for an additional 9 weeks. Of the patients who received the 106 U dose, 53% had complete clearing of their lesion compared with 17% of the placebo groups. Most responses occurred between 4 and 9 weeks despite the fact that injection was stopped at 3 weeks (Fig. 5).

Eron et al.⁹ reported a randomized, double-blind trial in which patients had as many as three warts injected with 10⁶ U per wart three times a week for 3 weeks.



Fig. 2. Laser vaporization at 1000 W/cm².



Fig. 3. Papillary dermis of the first surgical plane is seen after epithelium is wiped away.

Complete responses occurred in 36% of these patients compared with 17% in the placebo group. In this study, an open-label phase was used in which those patients who received interferon and responded but whose disease then progressed were retreated with interferon. Their response rates were similar to those of the originally treated group. This finding indicates that patients who respond to interferon and then suffer a recurrence can be expected to respond when treated again. It also suggests that treatment may be required beyond the currently recommended 3 weeks.

The use of interferon after laser vaporization has also been successful in reducing recurrences.6 At the site of laser treatment, 106 U of interferon is injected three times a week until complete healing has occurred. This treatment is particularly helpful in the treatment of patients whose warts are on the hair-bearing areas of the skin. Interferon is effective in eliminating regrowth of warts from the hair follicles, allowing the laser vaporization to be limited to the first surgical plane.

A 27-gauge insulin syringe is used for injection, because these syringes have no hub and all the medicine

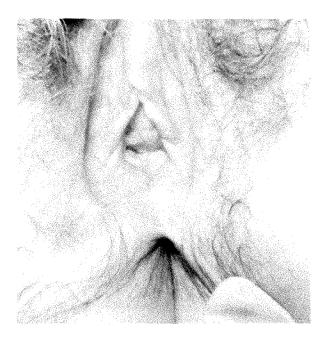


Fig. 4. Appearance of patient 10 weeks after laser treatment with adjuvant interferon used for 4 weeks to reduce recurrence.

is delivered. The injection is placed intradermally at the base of the wart or into the wart itself. The major side effect of interferon is a flulike syndrome. This occurs 2 to 4 hours after injection and abates 4 to 6 hours later. This syndrome responds to acetaminophen and decreases with each treatment so that patients hardly notice it after the first week. Hematologic effects at the 106 U, three times weekly dose are negligible, and a complete blood cell count is not routinely performed. Patients receiving 3×10^6 U three times weekly may experience leukopenia in the 3000 cells/mm³ range; a complete blood cell count should be obtained if treatment goes beyond 3 weeks. The leukopenia is reversible on cessation of treatment. Mild elevations in liver enzyme levels have been observed but are not of clinical significance, and monitoring is no longer performed.

Interferon is also very useful as an adjunct to acid treatment for the large or still-proliferating warts. It reduces the total time of treatment and the rate of recurrence.

Clinical implications of HPV infection

It is now recognized that genital neoplasia is a continuum that begins with HPV infection. Attempts have been made to correlate morphologic findings, HPV type, and histologic appearance. The implications of these studies are that the distinction between flat condyloma and true cervical-intraepithelial neoplasia (CIN) has important clinical relevance, and therefore

the management of these lesions may be different. Willet et al.¹⁰ studied 71 patients with lesions ranging from flat condyloma to CIN and analyzed them for HPV type and the presence or absence of abnormal mitotic figures. HPVs 6 and 11 were found in 5 of 25 of the flat condylomas and in 0 of 14 of the CIN IIIs. Conversely, HPV 16 was found in 12 of 14 of the CIN IIIs and in 6 of 25 of the flat condylomas. Abnormal mitotic figures were found in 4% of flat condylomas, 13% of CIN I lesions, 39% of CIN II lesions, and 64% of CIN III lesions. Because of the difficulties in histologic discrimination and similar distribution of HPV types in low-grade lesions, they proposed that the flat condyloma and CIN I be grouped together and the term "flat condyloma" be abandoned. The Bethesda System¹¹ of cytologic classification also recognizes that these lowgrade lesions are hard to separate and groups them together. The investigation of HPV subtypes in invasive cervical cancer has disclosed that HPV 16 is found most commonly in squamous keratinizing tumors, whereas HPV 18 is found more commonly in adenocarcinomas and poorly differentiated squamous carcinomas. In a study by Wilcynski et al.12 of 41 cervical carcinomas, HPV 16 was found in 77% of the large-cell keratinizing tumors and in one of eight adenocarcinomas. Conversely, HPV 18 was identified in four of eight adenocarcinomas and in two of 13 large-cell keratinizing tumors. Patients with the large-cell nonkeratinizing tumors are the highest percentage in whom no HPV deoxyribonucleic acid was identified (9/16). Barnes et al.13 studied 30 patients with invasive cervical carcinoma and found that five of six HPV 18-associated tumors were grade 3 compared with 7% of HPV 16-associated tumors. The HPV 18-infected group was significantly younger (average of 37 vs 49 years of age) and had a higher incidence (60% vs 36%) of lymph node metastases. This suggests that HPV 18 may be associated with a more aggressive form of cervical cancer. Kurman et al. 4 reported on overrepresentation of HPV 18 in invasive cancers when compared with CIN and suggested this as evidence that HPV 18 lesions may progress rapidly through the CIN stage to invasive cancer.

Vulva intraepithelial neoplasia (VIN) and invasive vulvar cancer are also associated with HPV. Twiggs et al. ¹⁵ studied 25 patients with either Bowen disease or VIN; 19 tested positive for HPV and 11 tested positive for HPV 16. Patients who were HPV positive were younger and had multifocal disease with involvement of several sites in the genital tract compared with those who tested negative for HPV. Buscema et al. ¹⁶ studied 86 patients with condyloma acuminatum, VIN, and invasive vulvar cancer. HPVs 6 and 11 were found in 77% of those patients with condyloma acuminatum and in 0% of those with VIN, whereas HPV 16 was found in 81% of those persons with VIN, 66% of those with

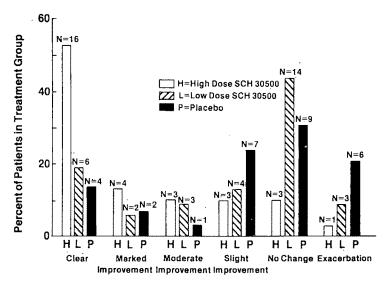


Fig. 5. Distribution of patients in disease status after treatment with 1 million U of α -interferon compared with 100,000 U and placebo. (From Colposcopy and Gynecologic Laser Surgery 1988;4:187-96.)

invasive cancer, and 12% of those with condyloma acuminatum. HPV 18 was found in a low number in both groups. Progression to invasive cancer from VIN has rarely been reported. Generally these patients have immunosuppression disorders, are over age 50 years, or both. Planner and Hobbs¹⁷ examined 335 patients by colposcopy who had cytologic evidence of HPV. They found that 94% had cervical infections, 82% had vaginal infections, and 44% (148) had vulvar disease. Of these 148 patients, 7.4% (11) had VIN. Two patients' disease progressed to VIN III, and one (age, 25 years) developed invasive cancer.

The most common mode of HPV transmission is sexual contact. Fifty percent of male consorts of women with condyloma acuminatum demonstrate visible lesions. Colposcopic examination after the administration of 5% acetic acid usually reveals a subclinical infection in an additional 24%.18 Barraso et al.19 reported 294 men who were consorts of women with flat condyloma acuminatum of the cervix. They found that 41.1% had HPV penile lesions and 3% had intraepithelial neoplasia. In addition, 186 men who were consorts of women with CIN were examined, and 36% of these men had penile intraepithelial neoplasis. Gal et al.20 found HPV lesions in 76% of the male consorts of women with HPV lesions with (n = 31) or without (n = 82) CIN. HPV typing of the male-female lesions disclosed homology in 60% of HPV 6 and 11 lesions and in 17% of HPV 16 and 18 lesions. Despite treatment of both men and women, a 10% recurrence rate occurred in the group with HPVs 6 and 11 and a 35% recurrence rate in the group with HPVs 16 and 18. This suggests that transmission by simple sexual contact is not the entire problem and barrier techniques, such as condoms, may not be effective.

HPV infection is increasing at an alarming rate. Its association with malignancy of the lower genital tract is the impetus to treat patients with infection. The ability of the virus to establish a latent infection and the lack of a specific antiviral agent make this a difficult task. Until such time as an antiviral agent is developed, the goals of treatment should be eradication of the obvious HPV infection and the intraepithelial lesions.

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Mycotic vulvovaginitis: A broad overview

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The incidence of mycotic vulvovaginitis is rising dramatically in the United States mainly because of an increase in infections caused by *Candida* species. Accurate diagnosis depends on culture techniques that will yield correct identification of fungal pathogen(s). Recurrences are common and require culture specimens from sexual partners and appropriate antifungal therapy. The imidazoles and more recently the broader spectrum triazoles are used for vaginal therapy. (AM J OBSTET GYNECOL 1991;165:1188-92.)

Key words: Mycotic vulvovaginitis, Candida species, fungal infections, antifungal agents

In the United States alone, 13 million cases of mycotic vulvovaginitis occur annually. During the past decade, the population of women 18 years of age and older has increased approximately 13%, whereas the number of prescriptions written annually for vulvovaginal candidiasis has increased more than 53%. In the United Kingdom, the number of cases of vaginal candidiasis reported in sexually transmitted disease clinics has increased from 21,000 in 1972 to 38,000 in 1982. Candida species were the third most frequently reported pathogens associated with nosocomial bloodstream infections as reported by Horan et al. in the National Nosocomial Infections Surveillance System between January 1985

and August 1988. This report indicates that in 1984 Candida was ranked eighth, thereby indicating a trend toward increasing prevalence of Candida. Interestingly, in the 1988 National Nosocomial Infections Surveillance System report, 35% of the yeast species were non-albicans.

These data and the daily experiences of clinicians affirm the impression that human mycoses in general and vaginal mycotic disease in particular are on the rise. The advent of immunosuppressive disease created iatrogenically with the use of cyclosporine in patients undergoing transplantation, pathologically in patients with acquired immunodeficiency syndrome, or chemotherapeutically in patients with cancer creates premature death not by the disease process but by the opportunistic infection by the lowly one-celled fungus—the yeast. It is evident that knowledge of and familiarity with fungi are of the utmost importance to physicians of all medical disciplines. As the human ex-

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Reprint requests: Benson J. Horowitz, MD, S.H.E. Medical Associates, 449 Farmington Ave., Hartford, CT 06105. 6/0/32261 perience evolves, association with the most primitive of organisms, the yeast, becomes more intimate. . .

Classification

The study of fungi has been complicated by their attempted inclusion into the plant or the animal kingdom. This confusion was created by the fact that some fungal processes are shared by both plants and animals. The dilemma was remedied by the establishment of a third kingdom called the kingdom of fungi. As a result, there are now three kingdoms: plants, animals, and fungi.

The taxonomic classification of the kingdom of fungi as it pertains to human disease is in the form phylum Deuteromycota, form class Blastomycetes, and form family Cryptococcaceae. The six genera of the form family Cryptococcaceae are of particular interest to the practicing vulvovaginologist. These genera are Cryptococcus, Malassezia, Rhodotorula, Candida, Trichosporon, and Geotrichum. Members of all of these genera have been found in women with vulvovaginal complaints. The most important members of the genus Candida in human clinical disease are: C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. pseudotropicalis, C. lusitaniae, and C. rugosa. C. stellatoidea has recently been incorporated in C. albicans, and Torulopsis glabrata was renamed C. glabrata.4 Currently the most frequent and important systemic and vaginal yeasts are C. albicans, C. tropicalis, C. glabrata, and C. parapsilosis.

In a random study population of women with vulvovaginitis caused by a fungus,5 C. albicans was found in at least 65% of patients. Twenty-three percent of these patients harbored C. tropicalis. In this study, Cryptococcus ungulaticus, Trichosporon beigelii, and Saccharomyces cerevisiae were also found. Three percent of the study population with vulvovaginitis had dermatophytes. In a study of 2184 isolates from 18 studies from 1963 to 1987,6 C. albicans was cultured in 84.2%, C. tropicalis in 5.3%, and C. glabrata in 5.5% of patients.

Morphology

Recognition of the morphology of fungal species leads to proper diagnosis. There are only three anatomic structures that the clinician must recognize for diagnostic purposes: the pseudomycelia, the blastospore, and the chlamydospore. The pseudomycelia is the long filamentous form with its arborization of branches. It is called a pseudomycelia because of its lack of segmentation. The blastospore is the collection of small refractile bodies resembling a group of glass beads, and the chlamydospore is a terminal birefractile sphere at the end of a pseudomycelia. Recognition of these structures is necessary to achieve therapeutic goals. Emphasis on filamentous forms versus budding forms is misplaced because depending on environmental conditions, the organism will assume either of these two anatomic structures. When the yeast assumes the filamentous form, candidal adherence is facilitated. The three candidal organisms of greatest prevalence, namely, C. albicans, C. tropicalis, and C. glabrata, are easily recognized by their morphology. The albicans organism consists of pseudohyphae, blastospores (blastoconidia), and chlamydospores (chlamydoconidia). The tropicalis organism has no terminal chlamydospore, and the organism appears as great clusters growing on a central stem. The glabrata organism contains no pseudohyphae and no chlamydospores but is only a collection of blastospores.

Biochemistry

The most important biochemical characteristics of yeasts are their ability to ferment and assimilate sugars. The specific sugar that is metabolized has been used as the traditional method of fungal identification. The tools of molecular biology offer more exact methods of identification; however, for the average clinician the former method is sufficient. The fermentation and assimilation patterns are easy ways to identify the particular species. Both five- and six-carbon atom sugars are metabolized. The efficiency of a yeast to convert sugars to carbon dioxide and ethanol cannot be duplicated by the chemist. Because of this, the brewing industry relies on the biochemical processes of the yeast in an anaerobic environment to make alcohol, and the baking industry relies on this process in an aerobic environment to leaven baked goods by the production of carbon dioxide.

In a study⁷ that used sugar chromatography,⁷ those cases of vulvovaginitis labeled recurrent or culture positive revealed a greater amount of sugar in the urine than in normal persons. From this work it was deduced that the amount of sugar substrate consumed by a woman was of importance in the continuation and persistence of vulvovaginal infections.

Pathophysiology and pathogenicity

The familiar reddening of the vaginal and vulvar tissues and the recognition of satellite lesions caused by candidal infection are easily recognized by the clinician. The pathogenesis of the reddening and the symptoms of itching, burning, and discharge are probably produced by the production of alcohol in the microaerophilic environment of the vagina. Other factors affecting the pathogenicity of the organism are sugar substrates;7 dressing patterns,8,9 antibiotic therapy,10,11 activated reservoirs, 12-14 antifungal bacterial factors, 15 adherence phenomena,16,17 immunologic hypersensitivity, 18-20 fungal species,5 and certain forms of contraceptives.21

Transmission of candidal organisms from a male partner to the vagina has been studied by several authors. 14, 22, 23 In a recent study of the male consorts of women who had culture-proved *Candida* vulvovaginitis, 33% had the same organism in their oral cavities, 36% in their rectal cavities, and 15% in their ejaculates. Results of prostatic cultures were negative; however, results of ejaculate cultures were positive. This suggests that the organisms reside in the seminal vesicles where fructose is abundant. In contrast, consorts of women without symptoms had negative results for cultured *Candida* orally and in their ejaculate. 14

Laboratory identification

The techniques for laboratory diagnosis of yeast infections have been simplified, and rapid diagnostic kits are available. Sabouraud's dextrose medium impregnated with chloramphenicol used as the basic culture medium for *Candida* is easily obtained from local suppliers. Thayer-Martin medium, an anaerobic culture medium used predominantly for the isolation of the gonococcus, may support the growth of yeast when Sabouraud's dextrose medium fails. After 5 days of incubation in ordinary conditions of light and temperature, the white mucoid colonies can be transferred to species identification kits. Incubation at 37° C with serum produces "germ tubes" (the Reynolds-Braude phenomenon). Germ tubes are produced by *C. albicans* and some species of *C. tropicalis*.

Just as it is necessary to identify bacteria because the individual characteristics of these organisms are important for therapy, so is it true of yeasts. Each of these yeasts have different characteristics. The nature of these differences will be of importance in the design of antifungal preparations and in the spectrum of recurrent and relapsing disease.

The use of culture kits provides the clinician with irrefutable evidence of a mycotic infection, indicates the exact species of the organism, provides information concerning sexual transmission, and delineates reservoirs. This information is essential in the treatment of recurring and relapsing disease.

Cell wall biochemistry and modes of action

Yeast organisms have a unique cell wall, and the biochemical pathways in the cell wall are important for its integrity. These pathways are more similar to those of the plant kingdom than to those of the animal kingdom. The sterol pathways proceed from squalene through squalene 2,3 oxide to lanosterol, and on to ergosterol. Antifungal agents are specifically designed to interfere with these biochemical pathways. The enzymes of the cytochrome P-450 group are particularly vulnerable, and this accessibility provides an opportunity for pharmacologic effectiveness. Yeast cell walls also consist of

the polysaccharides glucan, mannan, and chitin, protein, lipids, and in some cases capsular polysaccharides.²⁴ Mannoproteins are the main antigenic components of the cell wall.

Yeast cells also contain estrogen receptors in the cell wall. Yeasts do not proliferate without estrogen support.

Pathogen susceptibility

In 1972 Van Cutsem and Thienpont,²⁵ testing miconazole's effectiveness against various species of yeasts, found that it took 10 times more miconazole to eradicate *C. tropicalis* and *C. glabrata* than *C. albicans*.²⁵ Takada et al.²⁶ reported that the minimum inhibitory concentration of imidazole antimycotics, including econazole, miconazole, and clotrimazole, against *C. glabrata* was two to four times higher than that against *C. albicans* isolates. These data unequivocally support the concept that all species of *Candida* are not the same and that recurrence and relapse may be from ineffective concentrations of antifungal agents.

Aspects of selection

The prevalence of non-albicans species in the 1970s, as tabulated in nine studies, approximates 9.9%. In the 1980s, seven studies revealed a prevalence of 21.3%. During this same period, the incidence of *C. glabrata* increased from 4.6% to 6.7%, and *C. tropicalis* increased from 1.3% to 8.2%. Data from clinical studies demonstrated an increase in non-albicans species from 9% in 1971 to 14% in 1987. The increase in *C. tropicalis* has been the most dramatic.

Although the exact cause of this non-albicans selection is unknown, there is some evidence that antifungal therapy itself may be the cause. Redondo-Lopez et al.27 concluded that most patients in whom C. glabrata was isolated were treated previously with a multitude of antifungal agents and that iatrogenic factors select for the presence of C. glabrata. Kerridge and Nicholas²⁸ found that strains of C. glabrata were more resistant to the imidazoles than were C. albicans. They noted that C. glabrata is haploid and C. albicans is diploid. This may account for increased drug resistance. Horowitz et al.5 and Merz and Sanford²⁹ described strains of C. tropicalis with the ability to forego the $14-\alpha$ -demethylation pathway in the cell wall, making these organisms resistant to imidazole therapy. All these data suggest that the non-albicans species are more resistant to imidazole and polyene macrolide therapy and that the therapy itself created the non-albicans selection.

The second theory involves the use of shorter courses of imidazole therapy. Support for this theory can be found from the history of such infections in patients with neutropenia. Increased numbers of systemic infections caused by *C. tropicalis*, *C. glabrata*, and other

non-albicans species were related to the routine use of prolonged, low-dose prophylactic polyenes and imidazoles to prevent oral and systemic candidiasis. Takada et al.33 described replacement of initial C. albicans vaginitis with C. glabrata in patients treated with clotrimazole.

Therapy

Commonly used products that eradicate fungal infections are the imidazoles and polyene macrolides. These products interfere with the sterol chemistry pathways in the cell wall of the fungal cell. The imidazoles interfere with the $14-\alpha$ -demethylase enzyme in the conversion of lanosterol to ergosterol. Interference is also noted in side-chain cleavage, the desmolase reaction, and the 11B-hydroxylase enzyme. Polyene macrolides, such as nystatin, katamycin, and amphotericin B, also interfere with the formation of ergosterol. The general formula of an azole antimycotic is a pentene ring containing two nitrogen atoms and three carbon atoms to which side chains are added. The configuration of the side chain determines the nature of the imidazole compound. Examples of the compounds are miconazole, clotrimazole, tioconazole, ketoconazole, and butoconazole.

Not all imidazoles are equally effective against all species of yeasts. For example, C. tropicalis and C. glabrata are 10 times less sensitive to the action of miconazole than is C. albicans. Rhodotorula is particularly insensitive to the action of miconazole. For this reason, other agents have been devised and they fall into two classes: a new azole antifungal, the triazole, and the pyrimidines.

The development of the triazole compounds, specifically terconazole, addressed several of the difficulties of the imidazoles. A triazole is distinguished from an imidazole by the addition of a third nitrogen atom in the pentene ring. Attached to the ring is a side chain. The configuration of the side chain is designed to make the compound more lipophilic for better cell-wall penetrability. Although both imidazoles and triazoles interfere with the cytochrome P-450 isoenzymes, the triazole is more potent. This potency increases the susceptibility of C. tropicalis and C. glabrata to terconazole. The biochemical configuration also creates enhanced antifungal action at lower pharmaceutical concentra-

Although azole compounds interfere with cytochrome P-450 processes both in the fungal and mammalian vaginal cell, the chemical structure of the triazole makes it more selective. The ideal antifungal agent, one that would interfere only with the fungal cell, is as yet unattainable; however, the enhanced selectivity of the triazole results in less vaginal irritation.34.35 Greater selectivity also allows for the development of compounds of greater concentration over shorter periods of time.

In contrast to the azoles, a pyrimidine is a six-carbon atom ring with four carbon atoms, two unsaturated bonds, and two nitrogen atoms. The pyrimidine acts in a totally different way in that it is converted to 5-fluorouracil when carried into the nucleus of the fungal cell where it actively destroys deoxyribonucleic acid and ribonucleic acid helices.

Recurrence and relapse

Recurrent and relapsing fungal infections cause great frustration to both patient and care provider. The management of these cases requires accurate diagnosis, which is crucial to appropriate therapy. Unless culture techniques are available, misdiagnoses will be frequent. Anaerobic lactobacilli, Mobiluncus, and Actinomyces can be mistaken for fungi. Recurrent fungal vulvovaginitis requires culture specimens from all partners14, 36 and appropriate fungal therapy directed at the source of the contagion. Vaginal therapy includes the imidazoles and more currently the triazoles; oral therapy, clotrimazole oral troches or nystatin pastilles; gastrointestinal candidiasis, nystatin tablets or powder or ketoconazole; and for ejaculate candidiasis, ketoconazole or fluconazole tablets. Prophylactic therapy with ketoconazole during the first 5-days of the menstrual cycle for six cycles or daily for 6 months was effective in preventing the symptoms of candidal vaginitis. However, symptoms returned after discontinuation of therapy.11

The continued application of current knowledge and the new therapies being developed will significantly aid the clinician in the quest for complete therapeutic success.

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Mode of action of anti-Candida drugs: Focus on terconazole and other ergosterol biosynthesis inhibitors

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A large proportion of the presently available antifungal agents are claimed to derive their activity from interaction with the biosynthesis of ergosterol, the key sterol in most pathogenic fungi. An important target for the allylamines, naftifine and terbinafine, is the squalene epoxidase. Interaction with the epoxidation step results in a decreased availability of ergosterol and an accumulation of squalene. Although the squalene epoxidase is clearly the primary target for this class of antifungals, it still remains an open question whether the fungistatic or fungicidal effects originate from a decrease in ergosterol or squalene accumulation. Indeed, preliminary evidence suggests that squalene does not change the physicochemical properties of membranes. Much more is known about the primary and secondary effects of the azole antifungals, such as miconazole, ketoconazole, terconazole, and itraconazole. Most of the imidazole and triazole derivatives are highly potent and selective inhibitors of the cytochrome P-450–dependent 14α-demethylation of lanosterol (P-450_{14DM}). Their potency and selectivity are determined by the nitrogen heterocycle and to a much greater extent by the hydrophobic *N*-1 substituent. The triazole antifungals, terconazole and itraconazole, combine a high affinity for *Candida* P-450_{14DM} with an exceptionally low effect on mammalian cytochrome P-450. (AM J OBSTET GYNECOL 1991;165:1193-9.)

Key words: Squalene epoxidase, 14α -demethylase, cytochrome P-450, azoles, allylamines, morpholines

Mode of action studies on antifungals show how these agents interfere with a target in the fungus and highlight the differences between fungi and the mammalian host. Thus these studies may improve our knowledge of the biochemical systems in both parasite and patient. Furthermore, these chemical compounds can be used as tools to investigate the biochemical peculiarities of fungi.

Ergosterol is the most common sterol in *Candida* species and most pathogenic fungi where it plays a major architectural and functional role. Therefore it is not surprising that the ergosterol synthesis pathway has been recognized as a major target of antifungal agents. Indeed, most of the presently available antifungals are claimed to interfere with enzyme systems involved in the synthesis of this 24-alkylated sterol. For example, the allylamines inhibit the squalene epoxidase, the azole antifungals, the cytochrome P-450—dependent 14α -demethylase, and the morpholines interfere with the Δ^{14} -reductase and Δ^{8-7} -isomerase (Fig. 1).

It is the aim of this overview to discuss the molecular basis of anticandidal action of the squalene epoxidase and 14α -demethylase inhibitors.

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The allylamines, naftifine and terbinafine, and the thiocarbamates, tolciclate and tolnaftate, induce an accumulation of squalene and a concomitant decrease in ergosterol synthesis in fungal cells. This indicates that both the allylamines and thiocarbamates inhibit the squalene epoxidase (Fig. 1). In dermatophytes there is a clear correlation between the inhibition of this important enzyme of the ergosterol biosynthesis pathway and inhibition of growth. However, although the squalene epoxidases of Candida albicans and Candida parapsilosis are almost equisensitive to terbinafine (95% inhibition at terbinafine concentrations between 0.2 and $0.9 \,\mu \text{g/ml}$), the latter species is much more susceptible to growth inhibition. Furthermore, the epoxidase of Candida glabrata is, compared with that of C. parapsilosis, about three times less sensitive to this allylamine, whereas the minimum inhibitory concentration value is 250 times higher. 1 It should be noted that penetration problems do not seem to explain these differences, because activity against squalene epoxidase in intact cells and crude extracts of Candida cells is similar.2 However, poor penetration of the Candida cell membrane accounts for the lack of activity of the thiocarbamates against yeasts.3

Squalene epoxidase inhibitors

Ryder¹ speculates that fungal species differ in their sensitivity to a decreased availability of ergosterol and also in their reaction to the intracellular accumulation of squalene. Indeed, the filamentous form of *C. albicans*

Fig. 1. Reaction sequences involved in the synthesis of ergosterol and target sites for allylamines, thiocarbamates, azole antifungals, and morpholines.

is more susceptible than the yeast form, whereas ergosterol biosynthesis is almost equally affected in both forms. Filamentous fungi seem particularly susceptible to the allylamines and require only partial ergosterol biosynthesis inhibition for full inhibition of growth. Does the sensitivity to the accumulating squalene determine the susceptibility? *Candida* species can grow semianaerobically. Under this condition low amounts of ergosterol are formed and high levels of squalene are found.² Thus *Candida* seems to be able to tolerate high amounts of squalene. It has been speculated that filamentous fungi are more susceptible to squalene accumulation. However, of interest is the high radioactivity incorporated from carbon 14 acetate into squa-

lene by *Trichophyton mentagrophytes* grown under control conditions.⁴ Almost 24% of the total radioactivity measured in the lipid extracts is found in the squalene fraction; this is only two times less than the radioactivity incorporated into ergosterol.⁴ The presence of squalene did not prevent growth.⁴ This is not surprising because microgram amounts of squalene have been found in *Phytophothora cactorum*, a fungus unable to epoxidise and cyclisize squalene to lanosterol,^{5, 6} and Margalith⁷ hypothesized that in *Phytophothora* "squalene itself may be assuming the architectural role (bulk function?) of sterols, allowing vegetative growth." Differential scanning calorimetry of multilamellar vesicles of dipalmitoylphosphatidylcholine containing increasing

concentrations of squalene (10 to 35 mol %) has shown that squalene, even at 35 mol %, induced no significant change (<1° C) of the dipalmitoylphosphatidylcholine midtransition temperature or the enthalpy of melting (unpublished results). This suggests that squalene does not change the physicochemical properties of the membranes and thus differs from its cyclic products formed by the squalene cyclase, such as lanosterol (Fig. 1). Indeed, the addition of lanosterol (5 to 15 mol %) to dipalmitoylphosphatidylcholine multilamellar vesicles lowered the transition temperature slightly but had a major effect on the enthalpy of melting,8 suggesting that lanosterol interacts with the phospholipids present. Therefore it is still an open question whether growth inhibition and especially fungicidal activity are consequences of the decreased availability of ergosterol, squalene accumulation, or both. Thus although the squalene epoxidase is clearly a target for the allylamines, more studies are needed to clarify the molecular basis for their fungistatic and fungicidal activity.

14α-Demethylase inhibitors

Since the synthesis of miconazole and clotrimazole in the 1960s, a huge number of azole derivatives have become available. Some are used topically, whereas others are used both topically and orally. All belong to the class of 14\alpha-demethylase inhibitors. 9, 10 Their antifungal activity originates from binding to a cytochrome P-450 (P-450_{14DM}) involved in the 14α -demethylation of lanosterol (in Saccharomyces cerevisiae, C. glabrata, and mammalian cells) or 24-methylenedihydrolanosterol (in filamentous fungi, the yeast form of Histoplasma capsulatum, Cryptococcus neoformans, and a number of C. albicans isolates) (Fig. 1). It should be noted that contrary to C. albicans, C. glabrata uses lanosterol preferentially as substrate.

Before a discussion of the interaction of the azole antifungals with this key enzyme of sterol biosynthesis, we should look at cytochromes P-450, which are present in just about every phyla in which they have been sought. They are membrane bound (mitochondrial inner membrane and endoplasmic reticulum) in eukaryotes, whereas the prokaryote forms are soluble proteins. Cytochrome P-450s catalyze the synthesis or metabolism of a long list of key compounds. Examples are sterols (e.g., ergosterol and cholesterol), steroids (e.g., androgens and estrogens), bile acids, vitamin A, thromboxane A2, prostacyclin, and leukotrienes.11 They also play an important role in the activation of vitamin D and the metabolism of xenobiotics. Most of the cytochrome P-450s are classified as monooxygenases. This means that they catalyze the following reaction: $NADPH_2 + O_2 + RH \Rightarrow NADP^+ + H_2O + ROH$. In this reaction, RH represents a substrate (e.g., lanosterol) to be oxidized by the P-450 enzyme. The reaction

requires molecular oxygen of which one oxygen atom is inserted into the substrate, whereas the other oxygen atom is reduced to water. NADPH2 provides the electrons needed for the activation of this process. The active site of P-450 contains a ferric prosthetic heme group, the substrate binds to the protein moiety of the P-450, and the heme iron is reduced. Molecular oxygen is bound, reduced, and activated at this site (Fig. 2, A

The specific enzymatic function of the cytochrome P-450s, that is, to activate oxygen for insertion into a substrate, originates from the electronic structure of the heme iron linked to the thiol of a cysteyl residue of the P-450 protein moiety (Fig. 2, A). The typical absorption maximum at 450 nm of the reduced carbon monoxide-P-450 complex also originates from the specific interaction of the prosthetic group with the protein. The name of this hemoprotein originates from this property. Indeed, the name cytochrome P-450 describes a carbon-monoxide-binding pigment, which absorbs at about 450 nm.

As shown in Fig. 2, B, azole antifungals bind to the heme iron and compete in this way with oxygen binding and activation. This results in inhibition of the P-450catalyzed reaction. Because carbon monoxide also competes with oxygen for the same binding place, we can measure the interaction of an azole antifungal by adding it to a cytochrome P-450-containing microsomal or mitochondrial fraction, reducing the heme iron (with dithionite), and bubbling the suspension with carbon monoxide. Binding of the inhibitor to the heme iron will reduce absorption at 450 nm, and this decrease is a measure for the amount of inhibitor bound. For example, 50% inhibition of carbon monoxide binding to microsomal P-450 from C. albicans is obtained with nanomolar concentrations of the imidazole derivatives, miconazole and ketoconazole, and the triazoles, terconazole and itraconazole (Table I). Another triazole derivative, fluconazole, is a much less potent inhibitor of carbon monoxide binding, indicating that it has a lower affinity for the Candida P-450. This already indicates that the potency of an azole derivative is determined not only by its binding to the heme iron. It has been shown previously that the activity is determined, at least partly, by the hydrophobicity of the N-1 substituent (Fig. 2, B).12 For example, itraconazole and its imidazole analog form equistable complexes with C. albicans P-450; the less hydrophobic ketoconazole forms a less stable P-450 complex, and the stability of the complex is not increased by replacing the imidazole by a triazole (R42164).12

Comparing the amino-acid sequences of mammalian P-450(s) (e.g., those involved in steroid synthesis and metabolism) with that of C. albicans P-450_{14DM} shows less than 25% identical amino acids.13 Therefore it should

Fig. 2. A, Sketch of part of cytochrome P-450_{HDM}. Iron protoporphyrin and substrate (lanosterol) bound to a hydrophobic domain of the polypeptide chain are shown. The iron (Fe²⁺) is linked to the thiology a cysteyl residue (C) that is part of a conserved region of the polypeptide in C. albicans P-450_{HDM} H, Histidine; R, arginine; I, isoleucine; G, glycine. Molecular oxygen is attached to the ferrous heme iron. B, Sketch of azole (terconazole)–P-450 complex. Triazole ring is linked to the sixth position of the ferric (Fe³⁺) heme iron by way of N-3. N-1 substituent is bound to a hydrophobic binding site in P-450.

be possible to synthesize selective anti-Candida compounds. The selectivity of the azole antifungals may be determined by both the azole and the N-1 substituent of the molecule. Terconazole and itraconazole (triazole derivatives) are, as shown in Fig. 3, more selective affectors of the yeast P-450s than the imidazole antifungals miconazole, clotrimazole, and bifonazole. However, major differences in selectivity are found antifungals.

the topically active imidazole antifungals.^{10, 13} For example, clotrimazole and miconazole interact preferentially with the *Candida* P-450, whereas bifonazole has highest affinity for P-450(s) of the testicular microsomes from piglets (Fig. 3). Replacing the imidazole ring of ketoconazole with a triazole ring (R42164) slightly decreases the interaction with the microsomal P-450(s) from piglet testes.¹³ Fifty percent inhibition is

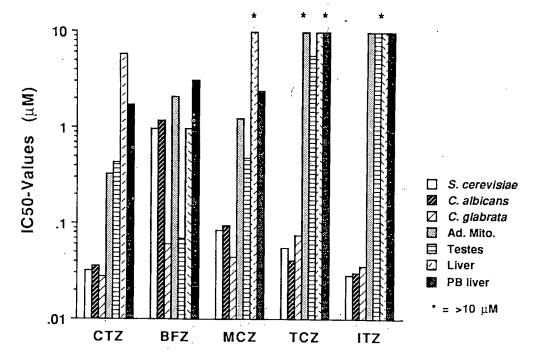


Fig. 3. Effects of clotrimazole (CTZ), bifonazole (BFZ), miconazole (MCZ), terconazole (TCZ), and itraconazole (ITZ) on microsomal P-450s from Saccharomyces cerevisiae (S. cerevisiae), C. albicans, C. glabrata, piglet testes, and liver from untreated and phenobarbital (PB) pretreated rabbits, and on mitochondrial P-450 from bovine adrenal glands (ad. mito). IC_{50} values = drug concentrations needed to inhibit carbon monoxide binding the heme iron by 50%. P-450 content = 0.1 nmol·ml⁻¹. Methods used are described in ref. 20.

Table I. Effects of azole antifungals on carbon monoxide-binding to microsomal P-450 and on ergosterol synthesis

	IC_{50} values (nmol/L)				
	P-450*		Ergosterol synthesis†		
Antifungals	C. albicans	C. glabrata	C. albicans	C. glabrata	
Miconazole	93.5	44.6	117	56.7	
Ketoconazole	31.1	30.4	60.3	52.8	
Terconazole	41.0	76.7	26.8	65.7	
Itraconazole	30.1	35.6	80.3	40.5	
Fluconazole	248.0	260.0	6530.0	1790.0	

IC₅₀ values = concentrations needed to achieve 50% decrease in ΔA (448 nm - 490 nm).

†Cells were first grown for 16 hours (8 hours in an orbital shaker) at 30° C in a polypeptone: yeast extract: glucose (10:10:40 & gmL/L) medium and then washed and resuspended in a 0.1 nmol/L potassium phosphate buffer containing 56 nmol/L glucose (pH 6.5). [4C]-Acetate, azole antifungals, and/or dimethylsulfoxide were added, and the cell suspensions were incubated for 2 hours at 30° C in an orbital shaker (300 rpm). At the end of the incubation period, sterols were extracted and separated by thinlayer chromatography. IC₅₀ values: Concentrations needed to obtain 50% inhibition of [14C]-incorporation into ergosterol.

achieved at 0.39 µmol/L (ketoconazole) and 0.49 µmol/L (R42164). Replacing the triazole of itraconazole with an imidazole ring slightly increased the effects on adrenal mitochondrial P-450 (from 0% to 25% inhibition at 1 μmol/L) but did not enhance the effects on testicular microsomal P-450.18 Furthermore, comparing the effects of ketoconazole with those of norketoconazole (deacylated ketoconazole) indicates that minor structural changes in the nonligand part (the N-1 substituent) affects the interaction with P-450. Norketoconazole has two- and three-times lower affinity for the microsomal P-450 from piglet testis and C. albicans, respectively. These results suggest that the nonligand hydrophobic part of the azoles has a greater impact on

^{*}Membranes used were isolated from C. albicans (ATCC 28516, microsomes) and C. glabrata (B 16205, microsomes).

		% of total of radioactivity	
	Sterol	Terconazo	
>>>		(0)	(1µM)
	Ergosterol	76.9	0.0
HO CH ₃	14α -Methyl-ergosta- Δ 8.24(28)-dien- 3 ß, 6α -diol	0.0	59.4
OH CH ₃	14α-Methylfecosterol	0.0	6.2
ich3	Obtusifoliol	0.0	8.2
HO Y Y	Lanosterol	0.0	5.9
HO CH ₃	24-Methylenedihydro- lanosterol	0.0	6.2
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Fig. 4. Effects of terconazole on ergosterol synthesis in C. albicans grown for 16 hours in casein hydrolysate, yeast extract, glucose medium supplemented with ¹⁴C-acetate and terconazole and/or dimethylsulfoxide. Homogenization of the cells, saponification, extraction, and separation of the sterols by high-performance liquid chromatography was described previously. [14C]-Acetate and drug (1 µmol/L) and/or solvent were added at inoculation. 0 = the control. Under the conditions used, an IC50 value of 45.9 nmol/L was found.

the selective interaction with P-450 enzymes than does the azole moiety and also prove that it is possible to synthesize highly potent and selective P-450 inhibitors.

The major P-450 present in C. albicans microsomes is involved in the 14α -demethylation of lanosterol or 24-methylenedihydrolanosterol, that is, P-450_{14DM}. Thus the effects of azole antifungals are suggestive of an interaction with the 14α-demethylase and thus with ergosterol synthesis. Inhibition of ergosterol biosynthesis has been shown with miconazole, terconazole, ketoconazole, itraconazole, and/or saperconazole in C. albicans, C. glabrata, Candida lusitaniae, Pityrosporum ovale, T. mentagrophytes, Paracoccidioides brasiliensis, Histoplasma capsulatum, and/or Aspergillus fumigatus.4, 8-10, 13, 14 The results listed in Table I show that after 2 hours of contact of C. albicans and C. glabrata with miconazole, ketoconazole, terconazole, or itraconazole, 50% inhibition of ergosterol synthesis from [14C]acetate is already reached at concentrations < 0.1 µmol/L. As could be expected from its lower effect on carbon monoxide binding to the microsomal C. glabrata and C. albicans P-450s, a much higher concentration of fluconazole is needed to reach 50% inhibition of ergosterol synthesis. Similar results are obtained after longer incubation periods.10 For example, complete ergosterol depletion is achieved when C. albicans is incubated for 24 hours with 0.1 µmol/L of itraconazole and ketoconazole, whereas 100% inhibition of ergosterol synthesis is not reached with 100 µmol/L fluconazole.10 It should be noted that ergosterol depletion is a prerequisite to block-cell proliferation.15

Inhibition of ergosterol synthesis coincides with the accumulation of 14-methylated sterols (Fig. 4). This proves that indeed the inhibition of ergosterol synthesis originates from interaction with the P-450-dependent 14α -demethylase. In the presence of terconazole, most of the radioactivity derived from [14C]-acetate is found in 14α -methyl-ergosta-8,24(28)-dien-3 β ,6 α -diol (3,6diol). The 6-hydroxylation may be part of the Δ^{5,6} double-bond insertion, one of the last reactions in ergosterol synthesis. The accumulation of this 3,6-diol in azole-antifungal-treated Candida may originate from the inability of the $\Delta^{5.6}$ -desaturase to use 14-methylsterols as substrates. Studies by Watson et al. 16,17 suggest that azole-induced growth inhibition originates in yeast from inhibition of the 14α-demethylase and the consequent accumulation of this 3,6-diol. Conclusive evidence has been collected showing that the 14-methylated sterols induce permeability changes, membrane leakiness, changes in membrane-bound enzymes, inhibition of growth, and cell death.8,9,18 For example, one of the most striking changes is an uncoordinated synthesis of chitin. Patches of chitin are found over the cell wall instead of being localized at the septa and growth tip. 14, 18 The accumulation of chitin disturbs the normal sequence of cell separation, resulting in chains and clusters of interconnected cells and causing abnormal swelling and bursting of cells.18 Other membrane-bound enzymes are also affected. The reduced nicotinamide adenine dinucleotide-dependent fatty acid desaturase requires the presence of a microsomal electron transport system that consists of reduced nicotinamide adenine dinucleotide cytochrome b5 reductase and cytochrome b5. All three components of the system are embedded in the microsomal membranes and affected by the fluidity of the membrane.19 It has been proven that the fatty acid desaturase of C. albicans is partially inhibited by the azole-induced (e.g., miconazole and ketoconazole) inhibition of ergosterol synthesis and the consequent decreased availability of ergosterol and/or accumulation of 14α-methylsterols.¹⁸ An inhibition of the fatty acid desaturase results in much higher levels of saturated fatty acids (e.g., palmitate) and thus in an increased membrane rigidity.20 This further contributes to the antifungal activity of azole antifungals.

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The in vitro activity of terconazole against yeasts: Its topical long-acting therapeutic efficacy in experimental vaginal candidiasis in rats

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The activity of the broad-spectrum triazole antifungal terconazole was evaluated in vitro by the serial decimal dilution technique in broth media. The best correlation between in vitro and in vivo activity was found in brain-heart infusion broth and Eagle's minimum essential medium. All strains of *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. glabrata*, and *Trichosporon beigelii* tested were susceptible. Terconazole blocked the morphogenetic transformation from the yeast into the filamentous form at concentrations of 0.008 to 0.05 µg/ml. In experimental candidiasis in castrated rats with estrogen-induced permanent pseudoestrus, topical treatment with terconazole was superior to miconazole, clotrimazole, econazole, butoconazole, tioconazole, sulconazole, bifonazole, valconazole, fenticonazole, nystatin, and amphotericin B in the various schedules used. A 3-day once-daily intravaginal application of terconazole 0.8% was usually sufficient to provide a functional therapeutic period of 7 days because of prolonged high biologically active antifungal levels in the vagina. No side effects were observed at any concentration of terconazole. (Am J Obstet Gynecol 1991;165:1200-6.)

Key words: Terconazole, anti-Candida activity in vitro, experimental vaginal candidiasis in rats, reference drugs, topical treatment, high antifungal levels in the vagina 4 days after therapy

During the past three decades, two major changes have occurred in the epidemiology of vaginal Candida infection: there has been a large increase in the incidence of the disease, and the percentage of non—Candida albicans infections has increased, especially C. (Torulopsis) glabrata.¹

With the introduction of nystatin in the 1950s, progress was made in topical therapy, but a more important step in the management of vulvovaginal candidiasis was the synthesis of the topical broad-spectrum imidazoles miconazole, econazole, isoconazole, and clotrimazole. Short-term oral medication became available with the introduction of ketoconazole, itraconazole, and fluconazole. However, topical treatment of this infection is still widely used, because safe and efficacious drugs for short-term treatment are available. This article deals with the in vitro activity of terconazole against yeasts responsible for vaginal mycosis. Its in vivo topical efficacy in experimental vaginal Candida infection in rats will be compared with that of other topical drugs.

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Reprint requests: Jan Van Cutsem, PhD, Department of Bacteriology and Mycology, Janssen Research Foundation, B-2340 Beerse, Belgium. 6/0/31516 Terconazole (R 42470)

CH₂

CH₂

CH₂

CH₂

CH₂

CH₂

CH₃

M.W.: 532.47 C₂₆H₃₁Cl₂N₅O₃

Fig. 1. Chemical structure of terconazole.

Material and methods

Terconazole (R42 470) is a broad-spectrum topical antifungal triazole derivative with a molecular weight of 532.47 (Fig. 1).²

In vitro experiments. The activity of terconazole was evaluated in broth media with serial decimal drug dilutions³ ranging from 100 to 0.01 µg/ml and drugfree controls. The sensitivity of several strains of

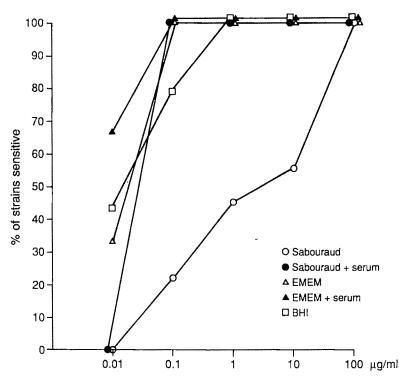


Fig. 2. Sensitivity of *C. albicans* to terconazole in broth media. Percentage of strains sensitive at stated concentrations.

C. albicans to terconazole was tested in tubes containing 5 ml of Sabouraud (n = 52), Sabouraud supplemented with 10% inactivated bovine serum (n = 3), Eagle's minimum essential medium (EMEM) supplemented (n = 3) or not (n = 3) with serum, and brain-heart infusion broth (BHI) (n = 28) (Fig. 2). BHI was used for strains of C. tropicalis (n = 26), C. krusei (n = 3), C. parapsilosis (n = 6), C. guilliermondii (n = 6), C. glabrata (n = 6), and Trichosporon beigelii (n = 6) (Fig. 3). The final concentration in the test media consisted of 2 to 4 × 10⁴ colony-forming units (CFU) per ml. The cultures were incubated for 2 weeks at 25° C and observed regularly. Final observations were made at the end of this period. The strains were considered sensitive if at least 90% of growth was reduced in the treated cultures versus the control cultures. To study the blocking of the morphogenetic transformation from the yeast into the filamentous form, four strains of C. albicans and one C. parapsilosis strain were tested with terconazole, miconazole, clotrimazole, and sulconazole (Table I). This experiment was performed in EMEM supplemented with nonessential amino acids and 10% fetal calf serum. The inoculum consisted of 150 CFU/ml. The drugs were diluted by fourfold serial dilutions at concentrations from 5 to 0.00064 µg/ml. The tests were performed at 37% C in a humidified atmosphere containing 5% CO₂. Cultures were observed for 7 days,^{4,5} and the presence or absence of hyphae was determined microscopically.

In vivo experiments. Female Wistar rats of 100 gm underwent ovariectomy and hysterectomy. After recovery, permanent pseudoestrus was induced by weekly subcutaneous injection of estradiol undecylate (1 ml of oily solution at 0.1 mg/ml). The rats were infected intravaginally with $8 \times 10^5 \text{ CFU}$ of *C. albicans*. They were treated topically twice daily for 3 days, starting 24 hours after infection with a volume of 0.2 ml of various concentrations of terconazole, miconazole, clotrimazole, econazole, nystatin, or amphotericin B (Table II). In other animals treatment with terconazole, miconazole, clotrimazole, butoconazole, tioconazole, sulconazole, bifonazole, valconazole, and fenticonazole was started 3 days after infection (Table III) and was administered twice daily over a 3-day period.

Rats with vaginal candidiasis were also treated with various azoles once daily, starting 3 days after infection for 3 consecutive days; some animals received one-shot therapy (Table IV). In all experiments excipient-treated animals (polyethylene glycol 200) were included. The evaluation was based on cultures in Petri plates from vaginal smears on Sabouraud agar supplemented with antibacterial antibiotics, on BiGGY agar, and on subcultures on agar media of vaginal swabs in Sabouraud broth tubes. The infection was checked 7

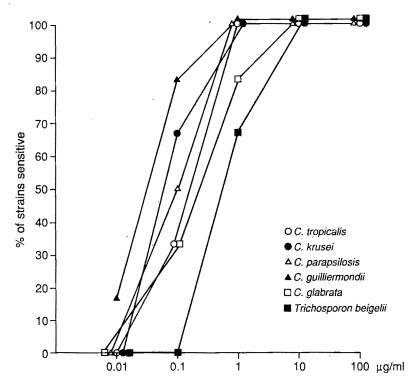


Fig. 3. Sensitivity of various yeasts to terconazole in BHI broth. Percentage of strains sensitive at stated concentrations.

Table I. Concentrations of azoles blocking the morphogenetic transformation from the yeast into the filamentous form of Candida

	Lowest concentration (µg/ml)							
	Terconazole		Miconazole		Clotrimazole		Sulconazole	
Strain	2*	7*	2	7 .	2	7	2	7
C. albicans ATCC28516	0.0016	0.008	0.2	0.2	0.2	0.2	1	1
C. albicans ATCC44858	0.0016	0.04	0.2	0.2	0.2	0.2	ND	ND
C. albicans ATCC44859	0.008	0.04	0.2	0.2	0.2	0.2	1	5
C. albicans B36215	0.0016	0.008	0.04	0.2	0.04	0.04	ND	ND
C. parapsilosis B34674	0.008†	0.008†	5†	5†	1†	5†	ND	ND

ND, No data.

†0.008: concentration that blocks the morphogenetic transformation of at least 80% of the yeast cells.

days after the last drug application. Animals were considered cured if all culture results were negative, and markedly improved if fewer than 25 colonies of *C. albicans* were present on the Petri dishes or if the subculture results from the swabs in the broth medium were positive. All others were considered treatment failures.

Bioassay study. The biologic antifungal level of terconazole was determined in the vaginas of rats after topical treatment once daily for 3 consecutive days with terconazole 0.8% in polyethylene glycol 200 or with the excipient (Fig. 4). Two groups of rats were used: (1) castrated animals in permanent pseudoestrus, which received weekly injections of estrogens and were infected with *C. albicans*, and (2) nonestrogenized, ovulating, noninfected rats.

Eight hours, 24 hours, and 4 days after completion of the treatment, six rats treated with the excipient or the drug were killed in both groups. The vagina and the uterus were removed and immediately deep frozen. For the bioassay experiment, the base layer of EMEM containing 2% of bacto agar was poured into Petri dishes of 140 mm diameter. Ten milliliters of seed layer, EMEM containing 0.75% of bacto agar, and 4×10^5

^{*2, 7:} days.

Table II. Topical therapeutic treatment of vaginal candidiasis in rats with various azoles (3 days twice daily starting 24 hours after infection)

		% of animals		
Drug and concentration (%)	No. of animals	Cured	Markedly improved	
Excipient	147	0	2	
Terconazole 0.063	20	25	15	
Terconazole 0.125	26	81	0	
Terconazole 0.25	24	79	8	
Terconazole 0.5	24	96	4	
Terconazole 1	12	100	0	
Miconazole 0.5	30	53	7∙	
Miconazole 1	38	63	5	
Miconazole 2	30	77	7	
Clotrimazole 0.5	24	29	13	
Clotrimazole 1	24	54	17	
Clotrimazole 2	24	63	25	
Econazole 0.5	12	25	0	
Econazole 1	18	56	11	
Econazole 2	18	67	17	
Nystatin 2	12	25	8	
Nystatin 4	6	33	ō	
Amphotericin B 2	6	0	Õ	

From Van Cutsem J, Van Gerven F, Zaman R, Janssen PAJ. Chemotherapy 1983;29:322-31. Basel, Switzerland: S Karger

CFU of C. albicans ATCC 28 516 per milliliter were poured uniformly over the base layer. Blank disks with a half-inch diameter were placed on the plates and dosed with various concentrations of terconazole in a volume of 0.05 ml. The vagina and the uterus were removed together, frozen, and then cut transversely, and the inner part was touched briefly onto the medium in one location and then placed on the surface in another. The test plates were left for 24 hours at 4° C to allow for prediffusion of drugs and then incubated for 48 hours at 25° C. The inhibition zones were measured, and the zone diameters were used to calculate terconazole concentrations in micrograms per milliliter or per gram value (Fig. 5).

Results

In vitro experiments. Terconazole proved to be highly active against C. albicans strains in vitro. The sensitivity of C. albicans to terconazole was more pronouced in long-term experiments in richer media compared with the classic Sabouraud broth (Fig. 2). In Sabouraud broth, the efficacy of the drug was rather poor, but in serum-supplemented medium, as well as in EMEM and BHI, drug efficacy was increased. The efficacy results with terconazole, obtained in BHI broth for C. tropicalis, C. krusei, C. parapsilosis, C. guilliermondii, C. glabrata, and Trichosporon beigelii, compared with those in Sabouraud broth, were in line with results for C. albicans (Fig. 3). Terconazole was able to block the morphogenetic trans-

Table III. Topical therapeutic treatment of vaginal candidiasis in rats with various azoles (3 days twice daily starting 3 days after infection)

		% of animals	
Drug and concentration (%)	No. of animals	Cured	Markedly improved
Excipient	188	0	1
Terconazole 0.125	64	38	8
Terconazole 0.25	60	57	20
Terconazole 0.4	20	75	15
Terconazole 0.5	80	81	4
Terconazole 1	46	98	0
Terconazole 2	16	100	0
Miconazole 1	27	33	22
Miconazole 2	44	52	11
Clotrimazole 1	20	25	20
Clotrimazole 2	24	46	13
Butoconazole 2	16	13	25
Tioconazole 2	16	25	0
Sulconazole 2	8	0	13
Bifonazole 2	16	6	13
Valconazole 2	8	13	13
Fenticonazole 2	6	17	33

Table IV. Topical therapeutic treatment of vaginal candidiasis in rats with various azoles starting 3 days after infection (1 or 3 days once daily)

Drug		% of animals		
Concentration %	No. of days once daily	No. of animals	Cured	Markedly improved
Excipient	3;1	40	0	0
Terconazole 0.5	3	16	56	25
Terconazole 0.8	3	12	92	8
Terconazole I	3	16	100	0
Terconazole 2	3	16	100	0
Miconazole 2	3	16	19	19
Clotrimazole 2	3	8	13	13
Butoconazole 2	3	8	0	25
Tioconazole 2	3	8	0	13
Terconazole 2	1	8	25	0
Terconazole 4	1	16	100	0
Terconazole 8	1	12	100	0
Miconazole 4	1	16	25	25
Miconazole 8	1	16	50	25
Clotrimazole 4	1	8	13	13
Clotrimazole 8	1	16	38	38
Butoconazole 8	1	8	0	38
Tioconazole 8	1	16	0	6

formation from the yeast into the filamentous form at concentrations of 0.008 to 0.04 µg/ml during the experimental period (Table I). Higher concentrations were needed for miconazole and clotrimazole, but these were much lower than those for sulconazole.

In vivo experiments. Vaginal candidiasis in castrated, estrogenized rats was a stable infection, and ex-

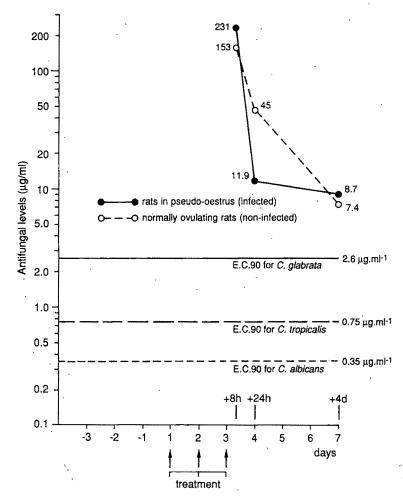


Fig. 4. Bioassay determination of antifungal levels of terconazole in vagina and uterus of noninfected and infected rats with C. albicans. Sensitivity of Candida strains.

cipient treatment had no influence on this condition. When topical therapy was begun 24 hours after infection and continued for only a short time (3 days twice a day), the first-generation, broad-spectrum imidazoles were active (Table II). However, nystatin was poorly active, and amphotericin B was not active at all. Of the imidazoles, miconazole was the most effective, but terconazole was far more potent.

When the onset of therapeutic treatment was delayed to day 3 after infection (Table III) and administered for 3 days twice daily, miconazole was the most efficacious of the imidazoles tested, followed by clotrimazole; the therapeutic results of the other imidazoles remained inferior. The therapeutic efficacy of terconazole was more pronounced at lower concentrations.

In therapeutic regimens, in which treatment started 3 days after infection and was administered either in a single shot or once daily for 3 consecutive days, terconazole was the most efficacious compound (Table IV). Side effects were observed in none of the terconazole-treated rats.

Bioassay. The antifungal levels in the vagina as measured by bioassay evaluation are presented in Fig. 4, and an example is shown in Fig. 5.

In both castrated, estrogenized, infected rats (group 1) and noncastrated, normally ovulating, estrogenized, infected rats (group 2), high levels of terconazole were detected after therapy. No inhibition was observed in the excipient-treated animals. Eight hours after the end of drug treatment, the levels per gram were 231 μg (SD 24) and 153 μg (SD 38) for groups 1 and 2, respectively. They decreased to 12 μg (SD 3.3) and 45 μg (SD 13) after 24 hours and to 8.7 μg (SD 2) and 7.4 μg (SD 1.4) after 4 days for groups 1 and 2, respectively. The concentration of terconazole present in the rat vagina 4 days after the last topical dosage was higher in all animals than the efficacy concentration of all yeast strains tested.

Comment

Terconazole is a potent broad-spectrum triazole. It is active against all yeast strains evaluted in vitro. In



Fig. 5. Bioassay in EMEM agar of vagina and uterus of rats treated with the excipient (upper left) and with terconazole 0.8%.

vitro results may be largely influenced by various factors.^{5,7} It has been established that the ingredients of the test medium play an important role in demonstrating the real potency of a drug. A good correlation between in vitro antifungal potency of terconazole and its in vivo efficacy was found by using BHI broth and EMEM. Both media support yeast development very well. BHI is a rich medium with a pH of 7.2 to 7.4, which allows higher concentrations of active nonprotonated drug. In EMEM supplemented with serum, the superiority of terconazole to other azoles tested was demonstrated by blocking the morphogenetic transformation of the yeast form into the filamentous form of *C. albicans* and *C. parapsilosis*.

Experimental vaginal candidiasis in ovariectomized and hysterectomized rats that were kept in permanent pseudoestrus by weekly administration of estrogens remained unchanged for at least 3 months. A small number of rats became spontaneously *Candida*-negative in a follow-up period of 8 months. In this model oral and topical therapy can be evaluated under the right conditions. Miconazole 2% must be administered twice daily for 5 days to obtain cure rates of 90%. For nystatin and amphotericin B, the treatment must be longer and

started very early (24 hours after infection) to eradicate the disease completely. The therapeutic efficacy of miconazole in the rat model has already been confirmed in the treatment of humans worldwide during the two decades of its use (first introduction of miconazole in human therapy was in 1971). Terconazole proves to be more active at lower concentrations. When the onset of treatment was delayed to 3 days after infection with a schedule of twice daily for 3 days, eradication of the disease was more difficult. Of all the reference substances, miconazole was the most potent, but a higher percentage of terconazole-treated rats was cured at lower concentrations. With the treatment schedule modified to once daily for 1 or 3 days, the superiority of terconazole over the reference substances was confirmed. At a concentration of 0.8% given once daily for 3 days or 4% given as a single shot starting 3 days after infection, 92% and 100% of the rats, respectively, were cured.

The high antifungal levels of 0.8% terconazole, administered once daily, in the vagina of infected, castrated, and noninfected, noncastrated rats that were still present 4 days after the end of therapy were usually higher than the inhibitory concentration needed for all

strains tested. This is especially important in view of the increasing number of non-albicans Candida infections. A short treatment schedule with terconazole 0.8% once daily for 3 days more or less covers a therapeutic period of 7 days for infections by the various yeast species, all causative agents of vaginal candidiasis. Cohen⁸ treated women with either 80 mg terconazole suppositories for 3 days or with 0.4% cream for 7 days once daily. The mean levels of terconazole in vaginal secretion 3 days after the end of treatment were 36 and 58 µg/ml, respectively. These concentrations were higher than those obtained in rats. The persistence of high levels of terconazole in the vagina is responsible for the complete eradication of the infection and for the reduction of risks for relapses. Such high concentrations of terconazole in the vagina allowed rapid elimination of yeast cells, subcellular changes in fungal cell membranes, complete necrosis of the fungi, and the absence of abnormalities in the vaginal epithelium as shown by scanning and transmission electron microscopy, confirming the safety of terconazole therapy in experimental animals.9, 10 Therefore the results obtained in the various experiments support the theory that terconazole is an excellent antifungal for topical therapy of human vaginal candidiasis in short schedules and has very low relapse rates.11-16

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Vulvovaginitis: The role of patient compliance in treatment success*

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Vulvovaginitis caused by Candida organisms accounts for a large number of annual office visits to physicians, often for recurrent infection. Despite the availability of many effective antifungal preparations, treatment failures continue to occur because of poor compliance with therapy. Several factors may foster noncompliance. Those involving the patient include denial of illness, misconceptions regarding the nature of the infection or its treatment, a misunderstanding of symptomatic relief versus microbiologic cure, dislike of the dosage form, nonsupport of the sexual consort, or intolerance of side effects. The cost of treatment, inconvenient dosage form, and prolonged duration of therapy may also contribute to noncompliance. This article offers suggestions for optimizing compliance and successful treatment. Two perceived means to this end are improved patient education and the use of short-term therapy in convenient dosage form. (AM J OBSTET GYNECOL 1991;165:1207-9.)

Key words: Patient compliance, vaginal mycoses, vulvovaginitis

The increasing frequency of vulvovaginitis has been traced to a number of diverse factors, including the widespread use of oral contraceptive pills and antibiotic administration. Whatever the underlying reasons, no one regards vulvovaginitis as a trivial disorder, because a large number of office visits to gynecologists are for the treatment of its symptoms. Such an overwhelming caseload translates into enormous morbidity among women and an ongoing challenge to clinicians.

A significant proportion of office visits for vulvovaginitis represent repeat visits by patients with chronic or recurrent infections despite the availability of seemingly effective therapeutic agents. Thus the question naturally arises, "Why are there so many therapeutic failures?" Aside from host factors, the most likely answer is poor patient compliance, which can compromise even the most effective treatment regimen. Hence the real challenge to physicians may not lie in diagnosing this condition, but rather in effecting better patient compliance.

This article explores the factors contributing to poor drug compliance and some of the options available to physicians for overcoming these problems.

Therapeutic regimen and compliance

It is generally held that the more precisely a therapeutic agent meets the needs of a specific patient, the

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more likley he or she is to comply with the prescribed regimen. The main patient factors to consider in selecting a therapeutic treatment for vulvovaginitis are listed in Table I. When such factors as dosage form and regimen, occupational or personal requirements, or prior patient experience with adverse effects such as burning or itching are taken into consideration by the clinician, the chances of patient compliance increase. For example, some patients may prefer the consistency of vaginal creams to suppositories or be less capable of complying with a 7-day regimen than a shorter regimen because of personal or business constraints. Thus the choice of therapeutic regimen can have a dramatic effect on compliance.

In general, the ideal therapeutic agent for vulvovaginitis is regarded as one that would (1) permit single-dose administration, (2) act rapidly, (3) be free of side effects, (4) be safe for use during pregnancy, and (5) offer a high long-term rate of efficacy. 1, 2 Problems with patient compliance have spurred efforts by pharmaceutical companies to improve treatment courses along these lines.

Psychosocial factors and compliance

Failure to comply with a full treatment regimen and subsequent treatment failure may stem not only from a less than optimal therapeutic choice but from numerous psychosocial factors. These are often a result of negative social influences and lack of information.³ These factors can include denial of the problem because of embarrassment, mistaking symptomatic relief for a cure, and in some cases even failing to understand anatomy and the nature of the infection. Another reason can be deferral to the preferences of a sex partner,

Table I. Patient considerations in choosing an antifungal agent for vulvovaginitis

Age
General health
Occupational requirements
Personal habits
Likelihood of compliance with instructions for use
Dosage form
Cost of treatment
Limitations imposed by concurrent therapy for other diseases
Prior experience with adverse effects

Modified from Jones HE. Med Clin North Am 1982;66:873-93.

who may find intercourse during treatment objectionable.

The cost of treatment can also profoundly affect compliance. This is especially true if friends or pharmacists inform the patient of less expensive over-the-counter preparations that are available. Because such information may discourage or alienate an otherwise cooperative patient, it is important for physicians to discuss their therapeutic recommendation in light of the availability of over-the-counter drugs.

Overcoming noncompliance

The chances for long-lasting resolution of symptoms and a microbiologic cure can be improved if physicians take a systematic approach to patient communication and education regarding dosing, side effects, and safety. It is essential that physicians take into consideration factors that influence convenience for the individual patient (Table II), because choosing regimens that control symptoms without disrupting daily life are more likely to be followed. For example, once-daily administration at bedtime has the advantage of facilitating drug retention, avoiding leakage onto daytime clothing, and coinciding with other nighttime rituals that afford a reminder to self-medicate.

In addition, choosing a short therapeutic regimen can minimize the opportunities for noncompliance. However, troublesome side effects can undermine even the most brief regimens. Fortunately, only a small percentage (≤7%) of women using the imidazole antifungal cream preparations experience adverse reactions such as pruritus, burning, or irritation, which also characterize the infection itself.⁵ It is important that physicians apprise their patients of these limited effects.

Pregnant patients can be reassured about the safety of treatment during the second and third trimesters, because its use has not been associated with any adverse fetal effects. However, imidazole and triazole antifungal drugs are not recommended for use during the first trimester, because embryotoxicity has been reported in some laboratory animals.

Table II. Factors influencing convenience of therapy in cases of vulvovaginitis

Factor	Recommendation
Daily timing of dose	Bedtime dosing
Frequency of administration	Once-daily administration
Length of treatment course	Short term (1-3 days)
Dosage form	Topical/oral agents
Use with menses	Advised for rapid response*
Sexual intercourse	Preferably abstain during short-course therapy or use condoms
Relation to regular daily routines	Follow above for no disruption of daily life

Modified from Sobel J. In: Sobel J, ed. Clinical perspectives: terconazole, an advance in vulvovaginal candidiasis therapy. New York: BMI/McGraw Hill, 1988.

*If menses remain a major barrier to patient cooperation, physicians should allow treatment to be postponed rather than risk noncompliance.

Cooperative decision making

Involving the patient in decisions regarding drug choices can go a long way toward improving patient communication and ensuring patient compliance. What may seem like a perfectly reasonable therapeutic approach to the physician may cause difficulties for the patient, and unless such matters are discussed, compliance may suffer. Fortunately, many aspects of treatment lend themselves to mutual decision making.

A patient's acceptance of a dosage form, for example, is governed by ease of use and the physical properties of the drug. Product consistency, the volume of application, color, scent, and applicator size all bear on the acceptability of therapy. Where possible, patients should be offered a choice of formulations, if there is no trade-off in efficacy or treatment duration.

Although sexual transmission of *Candida* vulvovaginitis remains to be definitively shown, I believe that sexual abstinence should be encouraged because it avoids compromising treatment and eliminates potential reinfection. Because effective treatment can often be achieved with a single dose or a 3-day regimen, sexual abstinence is usually not a problem and can be discussed cooperatively with the patient.

Education and communication

Misconceptions about the nature of vulvovaginitis and its treatment may also contribute to poor compliance with therapy. Thus patients should be told that treatment should be continued during the menses because topical medications are formulated to adhere to the vaginal mucosa even during menstruation. Further, discussing the effect of the menses on the vaginal pH and flora may dispel the reluctance of some patients to proceed with therapy. However, if it appears that menstruation is a major barrier to cooperation, it is better

to agree to the postponement of treatment rather than risk noncompliance.

Even when treatment has been optimal, the tendency exists for some infections to recur. Patients should be counseled about this possibility and informed about the nature of resistance. This can minimize the development of negative feelings about themselves, their therapy, or their physicians. Similarly, women must be instructed to continue therapy even after achieving symptomatic relief. This is vital, because many currently available agents provide relief within hours of initial use, and symptomatic relief does not necessarily equate with ultimate treatment success.

Therapeutic compliance is also enhanced when patients trust their physician and his or her staff. Women who develop confidence in the skills of the health-care team to provide a correct diagnosis and appropriate treatment are more likely to comply with the recommended regimen. Also, the more patients trust their physicians, the less likely they are to be influenced by family or friends who may have had a negative experience with similar therapeutic regimens. This trust can be fostered through displays of mutual respect, frankness, concern, and willingness to impart information and answer questions. Recent studies have shown that patients who are encouraged to ask questions of their physician not only do so but feel less anxious about their visit, are in better control of matters, and are more satisfied with both the visit and the information they received.6-9

Printed materials can also play an important role in promoting compliance because they allow patients an opportunity to review relevant information at their leisure. Moreover, these educational adjuncts enhance the perception of quality care.7

Finally, it is important to inform patients that despite the best efforts of all parties, vulvovaginitis may recur. When this occurs, it is the physician's responsibility to determine if faulty compliance, microbial resistance, or some other factor is to blame. Follow-up visits for second treatment should include continued patient education to help decrease the chances for noncompliance.

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Bacterial vaginosis: Current review with indications for asymptomatic therapy

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Bacterial vaginosis is a definable clinical entity whose exact origin is unknown. A shift in normal vaginal flora from aerobic, predominantly but not exclusively lactobacilli, to a predominantly anaerobic flora characterizes the condition. More than one half of all women with bacterial vaginosis have no symptoms. The condition is not entirely benign. The potentially pathogenic bacteria present in the vagina in large numbers place these women at risk for postoperative morbidity and adverse obstetric outcome. Sexual transmission has not been proved, but therapeutic cures sometimes require that patient and partner be treated simultaneously. Recommended therapy is with metronidazole or clindamycin and must be given for 7 days for maximal effectiveness. Recurrence of disease can be a problem. (AM J OBSTET GYNECOL 1991;165:1210-7.)

Key words: Vaginosis, vaginitis, vaginal flora, metronidazole, clindamycin

Bacterial vaginosis is currently the most prevalent infectious cause of vaginitis.1 The condition is characterized by a profuse, malodorous vaginal discharge, but more than one half of patients with demonstrable signs have no symptoms.2 Bacterial vaginosis has been known by various names. Before 1955, all types of vaginitis not caused by trichomonads, yeasts, or gonorrhea were called "nonspecific vaginitis." Gardner and Dukes^{8, 4} recognized that the condition was a specific entity, which they named Haemophilus vaginitis, and they believed that it was caused by a specific bacterium, Haemophilus vaginalis. In time the bacterium could not be considered taxonomically Haemophilus, because it had no absolute requirement for hemin, and thus it became Corynebacterium vaginale.5 The organism was moved eventually to a new genus, Gardnerella.6 It became evident over time that Gardnerella was not the only etiologic cause of bacterial vaginosis, largely because of the inability to eliminate Gardnerella in patients who were cured and its isolation from vaginal secretions in many women without bacterial vaginosis.7-9 Names for the condition reflecting the changing taxonomy of the bacterium were discarded: Haemophilus vaginitis, Corynebacterium vaginitis, and Gardnerella vaginitis.

Attention turned to anaerobic flora that were associated with bacterial vaginosis. ¹⁰ Anaerobic bacteria and not *Gardnerella* produced enzymes that were responsible for the distinct fish-like odor associated with the

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condition. Anaerobes produce aminopeptidases, which break down proteins into amino acids and decarboxylases, which convert amino acids and other compounds into amines; that is, decarboxylation of ornithine (a metabolite of arginine) yields putrescine, decarboxylation of lysine yields cadaverine, and decarboxylation of betaine (a metabolite of choline) yields trimethylamine. 11. 12 Recognition of the role of anaerobes led to the syndrome name of anaerobic vaginosis. 15 Eventually the current designation, bacterial vaginosis, was agreed on by international convention. 14

Microbiology of bacterial vaginosis

The normal vaginal ecosystem is very complex. The predominant bacterial flora are lactobacilli, but a large variety of both aerobic and anaerobic bacteria can also be present, many of which are associated with bacterial vaginosis when found in increased numbers. ¹⁵⁻¹⁸ For this reason, taking a vaginal culture is not a clinically useful diagnostic procedure. The numbers of bacteria in the normal vaginal ecosystem are 10⁵ to 10⁶/gm of secretion but in bacterial vaginosis are greatly increased to 10⁹ to 10¹¹/gm of secretion. ^{13, 19}

The cause of this shift in the vaginal ecosystem of women with bacterial vaginosis is not known. Normally occurring lactobacilli, especially the hydrogen peroxide—producing strains, become less evident.²⁰ Aerobic bacteria that produce succinate synergistically allow prolific growth of anaerobes.²¹ A single bacterium that inhibits lactobacilli and produces succinate to initiate the anaerobic overgrowth has not been identified; therefore *Gardnerella*, various anaerobes, and possibly genital mycoplasmas may have a role in the development of the full-blown syndrome.

Table I. Bacterial vaginosis incidence rates

Sexually transmitted disease clinics	-
Hallen	36%
Eschenbach	33%
Embree	64%
Hill	37%
Gynecology clinics	
Eschenbach	15%
Thomason	23%
Family planning clinics	
Hill	29%
Riordan	26%
Hill	23%
Obstetrics clinics	75.70
Gravett	10%-25%
Hill	23%
Thomason	26%
Asymptomatic college populations	
Éschenbach	4%
Symptomatic college populations	~70
Eschenbach	15%
Amsel	24%
Private physician populations	~ 170
Hill	16%

Incidence and risk factors

The incidence of bacterial vaginosis varies in different populations (Table I). Older studies showed rates of 30% to 45% in reproductive-age women in varied clinical settings, including sexually transmitted disease clinics, student health clinics, and private practice; the highest rate, 45%, was reported by Gardner et al.22 in women attending private practice. Modern reviews show a wide diversity of incidence, probably resulting from more exacting objective criteria used to diagnose the condition in women without symptoms. Rates in Sexually Transmitted Disease clinics range from 33% to 64%.1, 23-25 Rates in prenatal or obstetric clinics vary from 10% to 26% (Thomason, unpublished data).26.27 Eschenbach et al.1 reported a 15% incidence in a gynecology clinic in which college students saw a physician because of complaints of the lower genital tract but only a 4% rate in an asymptomatic college student population. Rates of 23% to 29% have been reported in other gynecology or family planning clinics.2, 28 However, lower rates have been reported in women seeking health care from private physicians (16%).27

A major risk factor for bacterial vaginosis is the use of an intrauterine device. 29-33 Barbone et al. 34 associated the number of different sexual partners within the month before examination as a risk factor for bacterial vaginosis. Other probable risk factors (e.g., age, smoking status, abnormal Papanicolaou smears, days of menstrual flow, age of menarche, years since menarche, use of a diaphragm, lifetime number of sexual partners, and rape) have not been associated with bacterial vaginosis. 29, 30, 35, 36

Table II. Sexual transmission of bacterial vaginosis

For	
Coital transmission of associated bacteria	
Correlation to number of sexual partners 30 days	
before examination	
Correlation to number of lifetime sexual partners	
Decreased rates in monogamous couples	
Different bacterial biotypes suggest newly acquired strains	
Lack of bacterial detection in virgins	
Against	
Bacteria do not persist in men	
Failure to show benefits of partner treatment	
Bacterial detection in virgins	_

Sexual transmission

It is not clear whether bacterial vaginosis is sexually transmitted. The literature provides evidence supporting both sides of the argument (Table II). Several authors have shown that bacteria associated with bacterial vaginosis, namely, Gardnerella, Mycoplasma, and Mobiluncus, can be recovered from urine and urethral scrapings of the male partners of women with bacterial vaginosis.4, 30, 37-42 However, the organisms do not appear to persist in men. An epidemiologic study searching for a possible reservoir or mode of transmission of the bacteria normally associated with bacterial vaginosis found that all such organisms could be isolated from the rectum in as many as 62% of women with bacterial vaginosis but only in about 10% of women without the disease.42 Holst also reported finding such organisms in the rectums of men and children. Studied organisms were recovered from the urethra or penile coronal sulci only in about one fourth of male consorts of women with bacterial vaginosis. After 2 weeks of condom use, only Mycoplasma hominis could be recovered from the urethra of one man. This study³⁰ supported earlier work in which Mobiluncus species were isolated from either the vagina or rectum of 29 (85%) of 34 women with bacterial vaginosis, which strongly suggests that the rectum may be a reservoir for this organism.

However, the role of the man as a reservoir cannot be ruled out from these studies, especially because a man may be recolonized as a result of unprotected intercourse with a different partner. Vejtorp et al.43 and Moi et al.44 found no beneficial effects on cure rates when partners of women having bacterial vaginosis are treated.43,44 However, criticism of such studies reports that failure was inevitable because of the use of tooabbreviated treatment regimens or because of a poor choice of antibiotic. Treatment of both patient and partner for a longer time span results in significantly higher cure rates, which suggests some relationship between bacterial vaginosis and sexual transmission.⁴⁵

Another argument against sexual transmission is the finding by Bump and Bueschling⁴⁶ that bacterial vaginosis could be detected in 12% of virginal women. This must be weighed against the report by Amsel et al.²⁹ of a university population in whom bacterial vaginosis was found in 69 (23.5%) of 293 sexually experienced women and in none of 18 who were virgins.

Evidence of sexual transmission comes from studies that show a strong correlation between the numbers of sexual partners 30 days before examination in women with and without bacterial vaginosis, greater than five lifetime sexual partners, and the decreased rates in monogamous couples. ^{27, 34, 47} Briseldon and Hillier ⁴⁸ recently showed that longitudinal biotyping of *Gardnerella* reveals women who acquire bacterial vaginosis are more likely to have *Gardnerella* strains with different biotypes than women who still had normal vaginal flora at their follow-up visits. This suggests that the *G. vaginalis* isolates recovered from women represent newly acquired strains rather than overgrowth of previously colonizing biotypes. Unequivocal sexual transmission of bacterial vaginosis remains to be proved.

Diagnosis

Malodorous vaginal discharge is the only symptom consistently reported by women having bacterial vaginosis. However, more than one half of all women with bacterial vaginosis may be entirely without symptoms, just as women may be without symptoms with infections caused by *Chlamydia trachomatis* or gonorrhea.⁴⁹

The clinical criteria developed by Amsel et al.²⁹ have become the reference standard for clinical signs of bacterial vaginosis. This group incorporated the observations of Gardner and Dukes,⁴ who described (1) the clue cell, (2) homogeneous discharge that adheres to but is easily wiped from the vaginal walls, and (3) an elevated vaginal pH, with the observation of Pheifer et al.³⁷ who developed (4) the potassium hydroxide test for volatile amines. The presence of any three of these four signs is considered diagnostic for bacterial vaginosis.

Recent studies of Thomason et al.⁴⁹ and Ceddia et al.⁵⁰ have shown that use of only two of the four objective signs (e.g., clue cells and positive amine test) allows accurate and rapid diagnosis of bacterial vaginosis without sacrificing sensitivity. The amount and physical appearance of vaginal discharge are difficult to evaluate objectively.⁵¹ The amount of discharge in women with bacterial vaginosis generally does not exceed that normally seen and can be affected by douching or recent intercourse. Vaginal pH is considered the most sensitive but least specific characteristic of the Am-

sel criteria, because it can be influenced by many factors, such as vaginal bleeding, douching, and recent intercourse.⁵¹ Clue cells, squamous vaginal epithelial cells so covered with attached bacteria that the borders are obscured, are the most sensitive and specific sign of bacterial vaginosis, especially when seen in wet mount preparations of vaginal secretion.⁴⁹ However, the use of clue cells as the single diagnostic criterion is limited to skilled microscopists.

Culturing for Gardnerella to make the diagnosis of bacterial vaginosis, although heavily relied on by earlier investigators, has in subsequent times proved worthless.^{1,52} Using specially designed media to enhance isolation,⁵³ it has been possible to find G. vaginalis in more than 50% of women with no bacterial vaginosis.¹ Even when Gardnerella is isolated in larger quantity, the positive predictive value for having bacterial vaginosis is only 40%.¹ Culture of Gardnerella is not useful for "test of cure," because many women without bacterial vaginosis have positive test results for this organism after effective treatment.⁵¹

Mobiluncus is a fastidious, curved, anaerobic motile rod more useful as a marker for disease than is Gardnerella.⁵⁴ The organism is highly specific for bacterial vaginosis but can be difficult to identify in wet mount examination of vaginal secretions because of its physical size.⁵⁵ Because of the difficulty in isolating this organism by culture techniques, genetic probe and monoclonal antibody immunofluorescent methods of identification have been developed.⁵⁶⁻⁵⁸ Virtually all women having Mobiluncus species identified in vaginal secretions have bacterial vaginosis.²³ However, not all women with bacterial vaginosis have Mobiluncus species as part of their abnormal vaginal bacteriology.

Bacterial vaginosis can be diagnosed by Gram's stain of vaginal secretions. Two methods of interpretation have been developed. The newer method of Nugent et al.⁵⁹ is more specific for bacterial vaginosis than the older method of Spiegel et al.⁶⁰; however, neither considers clue cells in its methodology. It is possible to recognize clue cells on Gram's stain. Combining the presence of clue cells with diminished lactobacilli morphotypes on Gram's stain may further simplify interpretation of such vaginal smears (Thomason JL, Gelbart SM, Osypowski PJ, unpublished observations). Clue cells and changes in flora can also be identified on Papanicolaou smears,^{61,62} but this has not proved as specific for bacterial vaginosis as other methods of identification.⁶³

Other rapid diagnostic methods for the identification of women having bacterial vaginosis have been developed but are largely relegated to research purposes. These tests are based on detection of bacterial metabolic products in vaginal secretions of women with bacterial vaginosis. Gas-liquid chromatographic analysis to detect succinic acid,10 thin-layer chromatography for the determination of putrescine and cadaverine,11 and enzymatic analysis for proline aminopeptidase2. 64 are three such tests.

Sequelae

Although bacterial vaginosis frequently produces few patient symptoms, serious infectious sequelae occur in women who have this disease. The following bacteria associated with bacterial vaginosis are known to be potential pathogens: Prevotella bivia, Prevotella disiens, Prevotella melaninogenica, G. vaginalis, and M. hominis. In addition, patients with bacterial vaginosis have greatly lowered vaginal tissue redox potential and elevated vaginal pH, both conditions known to be associated with increased infective potential.65 In addition, enzymes and metabolic by-products of the abnormal bacterial flora significantly impede normal white blood cell response to infection.

In the patient with gynecologic disease, bacterial vaginosis is associated with laparoscopically proved pelvic inflammatory disease, urinary tract infections, endometritis, and postoperative vaginal cuff infections. 35, 66-70 First suggested by Westrom 66 and confirmed by Paavonen et al.67 laparoscopically proved pelvic inflammatory disease is rarely seen if the vaginal secretions show no clue cells and if lactobacilli are the predominant flora. Colonization of the vaginal introitus as seen in bacterial vaginosis increases the risk of urinary tract infections. 85,68 Faro et al.71 relate bacterial vaginosis to postoperative infections in both obstetric and gynecologic settings. Cervical dysplasia has also been linked epidemiologically but not causally with bacterial vaginosis (relative risk 2.0).71a

In patients with obstetric disease, bacterial vaginosis is related to preterm labor, premature rupture of membranes, chorioamnionitis, and postcaesarean and postpartum endometritis.71-74 Literature before 1989 was reviewed by Martius and Eschenbach,75 who discussed how phospholipase A2 production could trigger the prostaglandin cascade leading to labor. Some bacteria associated with bacterial vaginosis are strong phospholipase A2 producers.76 McGregor et al.77 defined multiple associations between various maternal reproductive tract infections and other known noninfectious risk factors and obstetric outcomes. With bacterial vaginosis as a single risk, the relative risk of preterm labor was 2.6 times normal. However, when bacterial vaginosis was combined as a risk with the finding of Mobiluncus on Gram's stain or the isolation of Mycoplasma from vaginal flora, relative rates of preterm birth increased sixfold from normal rates. When combining the wellknown risk of a history of a previous preterm birth with other infectious risks, population-attributable risks for preterm birth reached 71%.

Treatment

Before discussing the therapeutic options, it is important to determine which patients with bacterial vaginosis should receive medical treatment. It is generally agreed that patients who do have symptoms should be treated to alleviate discomfort, but more than one half of patients with bacterial vaginosis do not have symptoms. The Centers for Disease Control recommendation that patients without symptoms should not be treated does not consider the implications of the more recently published studies documenting serious infectious sequelae.78 The explosion of papers on bacterial vaginosis in recent years and the question of sexual transmission of this disease make such guidelines difficult to formulate. Risk from therapy versus benefit to patients must always be weighed. When risk is low from antibiotic therapy and only the patient must be considered, recommendations are easier to formulate. However, when both a mother and an unborn infant are involved with therapy, guidelines are more difficult to recommend. Currently data from sequelae are strong enough to warrant treatment without hesitation in some asymptomatic populations.

Asymptomatic gynecologic patients with bacterial vaginosis who are about to undergo outpatient ambulatory invasion procedures, such as endometrial biopsy, hysteroscopy, hysterosalpingography, or placement of an intrauterine device, should be treated before these procedures. Women with bacterial vaginosis scheduled for vaginal or abdominal surgery should also be treated before surgery. We recommend treatment of this population with a full course of therapy and not just a prophylactic dose of antibiotics immediately before the procedure. By reducing the large number of potentially pathogenic bacteria, it should be theoretically possible to reduce the complications of postoperative infections. 79 This is a controversial area because only recently has bacterial vaginosis been proved a risk factor for various postoperative complications. Therefore the modulating effects of preoperative or perioperative prophylactic antibiotics have not yet been proved.

Treatment of obstetric patients who have bacterial vaginosis is more controversial. Patients with premature rupture of membranes or a history of premature labor may benefit from therapy. As elucidated by McGregor et al.,77 the risk of adverse obstetric outcome because of bacterial vaginosis is low; however, when coupled with other factors, the risk becomes great enough to consider strongly treatment of this underlying condition.

Interestingly, Bump et al.80 have demonstrated a high

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Table III. Antibiotic therapy for bacterial vaginosis

Effective Metronidazole, 500 mg, orally twice daily \times 7 days Clindamycin, 300 mg, orally twice daily × 7 days Clindamycin 2% intravaginal cream (research) Metronidazole 0.75% intravaginal gel (research) Questionable Ciprofloxacin Amoxicillin/clavulanate potassium Metronidazole, single 2 gm dose Triple sulfa cream Lactate gel Ineffective Erythromycin Tetracyclines Ampicillin Povidone iodine Acetic acid Dienestrol

spontaneous cure rate in some patients without symptoms. For this reason, placebo control groups are recommended for antibiotic effectiveness trials. Antibiotic treatment of bacterial vaginosis can also lead to vulvovaginal yeast infections. 81 Each health care professional should advise patients, whether they do or do not have symptoms, if bacterial vaginosis is found during a pelvic examination. Risks and benefits can be weighed by patients, who should actively participate in the decision about the need for therapy.

Therapy of bacterial vaginosis has recently been reviewed by Hillier and Holmes⁵¹ and by Lossick.⁸² Metronidazole and clindamycin are the most effective antibiotics (Table III). Erythromycin does not work, because macrolide antibiotics are not effective at the acid pH level of the vagina.88 Ampicillin does not work, probably because of poor activity against anaerobes, especially with production of β-lactamases.84 Other newer antibiotics, such as ciprofloxacin85 and amoxicillin/clavulanate,86,87 show some effectiveness but appear to be less efficacious than metronidazole or clindamycin. Ventolini et al.88 have shown that twice-daily douching with various preparations, including povidone iodine and aluminum acetate, does not effectively cure bacterial vaginosis. Povidone iodine, even when inserted as a vaginal pessary twice daily for 14 days, was completely ineffective.89 Other nonantibiotic therapies, including acetic acid gel, dienestrol cream, and yogurt preparations, have not been found to be efficacious; however, a newer lactate gel, studied in Europe, has been reported to have a high cure rate. 90, 91

Metronidazole, when used systemically, should be given for at least 7 days. Metronidazole used in a shorter or immediate 2 gm dose gives lower cure rates both initially and in longer follow-up studies. ⁵¹ However, systemic metronidazole therapy may not be necessary. Intravaginal treatment with 500 mg of metro-

nidazole twice daily for 7 days has been found to be effective. ⁹² Vaginal sponges impregnated with metronidazole have also been shown to be efficacious. ^{93, 94} More recently a metronidazole gel preparation used for 5 days looks promising but is available only through research protocol.

Clindamycin, when used systemically, should be given for 7 days. Although a 300 mg twice daily dose has been found effective, higher cure rates may occur if a three times per day regimen is used (Thomason, unpublished data). Topical administration of clindamycin has also been effective for bacterial vaginosis. A 2% clindamycin cream preparation used once daily in a 5 gm dose has a cure rate of 94%. Longer term (28 to 35 days after therapy) cure rates were in the 90% range. This drug formulation will be marketed in the near future.

Sexual partner therapy

As discussed previously, sexual transmission of bacterial vaginosis has not been proved, but coital transmission of associated microorganisms occurs. This creates a therapy paradox. To date data show no benefit of partner therapy if clinical signs were used to define cures. However, one study using more objective diagnostic criteria demonstrated that treating partners significantly increased cure rates in women. Although current recommendations from the Centers for Disease Control are that routine treatment of sexual partners is unnecessary, women who have intractable disease or recurrent bacterial vaginosis should be counseled about the possible benefit if partners also receive therapy.

Recurrence

Recurrent disease is a serious clinical problem. Hillier and Holmes⁵¹ anecdotally refer to an 80% recurrence rate within 9 months of completion of metronidazole in their studies. Although reasons for recurrence are not understood, they give the following possible explanations: (1) reinfection by a male partner who is colonized with bacterial vaginosis—associated microorganisms; (2) recurrence as a result of the persistence of bacterial vaginosis—associated microorganisms that are inhibited but not killed during therapy; (3) the failure to reestablish the protective *Lactobacillus*-predominant flora after therapy; and (4) the persistence of an unidentified host factor, which makes the woman susceptible to recurrence.

Conclusion

The pathophysiology of bacterial vaginosis remains inexact. The effects of specific antibiotic therapy on the vaginal bacterial population are largely unknown. It has taken decades to define normal vaginal flora and to realize that women having bacterial vaginosis may be

without complaints of vaginal discharge. Earlier studies frequently defined the control population as one "without symptoms." Diagnostic criteria used objectively to identify women having bacterial vaginosis without observer bias continue to be defined. Serious sequelae documenting the predisposition to infectious morbidity in women with this disease have only been identified recently. Trials to show the value of therapeutic intervention in women with and without symptoms can now be justified.

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Trichomoniasis: Trends in diagnosis and management

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The mainstay of the diagnosis of trichomoniasis has been the saline vaginal wet preparation. With a less than desirable sensitivity, the wet preparation may be replaced in the near future by newer methods employing monoclonal antibodies, such as the enzyme immunoassay, which has the potential to become an in-office procedure. The direct fluorescent antibody test also represents an advance in laboratory diagnosis. However, until the sensitivity, specificity, and cost of these newer techniques are defined outside the research arena, the wet preparation will remain the first-line diagnostic tool. Current treatment of trichomoniasis in the United States is with metronidazole, which in repeated or increased dosage can often overcome the organism's resistance to the drug. Other treatments offer little or no chance for cure but may provide some relief of symptoms. Tinidazole (not available in the United States) may be effective in curing refractory cases of metronidazole resistance. Metronidazole treatment during pregnancy should be resorted to only when absolutely essential. (Am J Obstet Gynecol 1991;165:1217-22.)

Key words: Trichomoniasis, wet preparation, Papanicolaou smear, culture, nitroimidazoles, metronidazole

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Trichomonas vaginalis is one of the most common organisms causing vaginitis in women worldwide and affects approximately 3 million American women annually.^{1,2} Although the older literature erroneously refers to *T. vaginalis* as a harmless commensal,^{3,4} its role as a pathogen is now universally accepted. For the most

Table I. Comparison of tests for diagnosing trichomoniasis

Technique	Advantages	Disadvantages
Vaginal saline wet mount	Low cost, easy, stat office diagnosis; 100% specificity if motile organisms noted	Low sensitivity by average microscopist (45%-60%)
Stained slide preparation (Papanicolaou smear)	Slide can be fixed and read/reread later; good sensitivity in skilled hands	Imperfect specificity even in skilled hands
Culture	"Gold standard"; 92%-95% sensitivity; can be used for susceptibility studies	Not readily available; more expensive than wet preparation; not a stat diagnosis
DFA	Transportable, stable; 80%-95% sensitivity; fast turnaround diagnosis	Not readily done in clinical setting; more expensive than culture; some false-positive results occur—specificity 99%-99.5%
EIA	Transportable, stable; good sensitivity; may be in-office test; selectively stains organism	Sensitivity lower than with culture; sensitivity and specificity need more definition; required costs and expertise?
Latex agglutination	Stat diagnosis; more sensitive than wet preparation (≤95%); can be run in batches	Lower sensitivity than culture; sensitivity and specificity need more definition; unknown costs; equivocal agglutination endpoint; not available in United States

part, *T. vaginalis* is considered to represent a classic venereal disease, despite its slight potential for non-venereal transmission.⁵⁻⁷

As in other infectious diseases, the diagnosis of trichomoniasis has been greatly influenced by the development of monoclonal antibody laboratory tests. A review of the various diagnostic procedures for making the clinical diagnosis of trichomonal vaginitis follows.

Diagnosis of trichomoniasis

Six tests are currently available to the clinician for determining the presence of vaginal trichomonads (Table I).

Vaginal saline wet mount. In use for more than 150 years and long the mainstay for the diagnosis of trichomoniasis, the saline wet mount of vaginal secretions has only recently been subjected to scrutiny as to its sensitivity and specificity. Approximately 60% to 70% of infections will be detected with this method.2 However, when compared to sensitivity of culture, sensitivity of the wet mount ranges from 45% to 60% in the hands of the average microscopist and from 60% to 92% in those of a highly trained researcher. Also, there is minimal quality assurance of interpretation of results of this procedure. In addition to the limited sensitivity of this test, another disadvantage is the need for immediate microscopic evaluation. As a plus, this test is inexpensive and has a virtual 100% specificity when motile organisms are identified.

When inoculum is adequate, *T. vaginalis* is easily identified by its pear shape, five flagella, and peculiar twitching motion. It can be seen, with its characteristic undulating flagella, darting between other nonmotile cells. Unfortunately, a negative wet mount in a patient with

acute florid vaginitis does not rule out trichomoniasis, since this organism can produce a soluble exotoxin that can cause inflammatory tissue reaction out of proportion to the number of organisms involved.

Stained slide preparation (Papanicolaou smear). Clinicians have long accepted the diagnosis of "trich" by the cytologist as part of their interpretation of Papanicolaou smears, and this has essentially been a "nocharge" benefit of routine cytologic procedures. One advantage of stained slide preparations like the Papanicolaou smear is that permanent preparations can be kept and reviewed later. Unfortunately, as with the vaginal wet mount, the specificity and sensitivity of this procedure have been determined only in the past decade. In an experienced cytologist's hands, this test has a similar sensitivity to that of the wet mount and a 99% specificity. Among less skilled technicians, however, its specificity approximates 95% to 99%.8 It is important that the cytologist maintain strict standards for objective determination of Trichomonas in the Papanicolaou smear.

As with the wet mount, there has been little quality assurance of Papanicolaou smear interpretations until recently, when some laboratories undertook concomitant culturing of vaginal smears as a reference check. Centers for Disease Control (CDC) studies have found that without routine culturing, both clinicians and technicians have no way of knowing if the sensitivity or specificity of diagnostic tests in their laboratories is appropriate. Routine culturing can provide cytologists essential information to improve their diagnostic skills.

With cytologic staining, as with any diagnostic procedure, one must remember that the positive predictive value will increase as the disease frequency increases in the population. Also, stained Papanicolaou smears, which are used mainly for screening women who have no symptoms, will exhibit sensitivity and specificity that are less than when employed as a diagnostic test in patients with symptoms. Since the load of the infectious agent is higher in the patient with symptoms, this and the wet mount will demonstrate a greater sensitivity in those women who have symptoms. Therefore, unless there is a high index of suspicion for trichomoniasis, preliminary results of cytologic tests must be confirmed before treatment is initiated.

Despite some putative improvements in staining procedures (acridine orange, Romanovsky, Diff-Quick, Harleco, Herstal, Belgium) and the development of dark-field and phase-contrast microscopy, none of these techniques has been used on a widespread basis.

Culture methodology. Possessing the ability to amplify the number of trichomonads in the original specimen and a high degree of sensitivity (92% to 95%), culture techniques are regarded as the "gold standard" in the diagnosis of *Trichomonas* infection. An added benefit is the ability to perform susceptibility studies on specific strains by use of these culture techniques.⁴

Unfortunately, routine diagnostic culture is not readily performed in the United States. Whereas many types of culture media are available, Diamond's TYM (tripticase, yeast, maltose) is regarded as the superior medium. Since trichomonads grow best under anaerobic conditions, the incorporation of 0.05% to 0.1% agar into the medium allows for superior growth because it reduces the diffusion of oxygen into the medium.⁴

The down side of culture techniques is threefold: the relative nonavailability of the media, the greater cost of the procedures, and the need to allow 3 to 7 days to elapse before the diagnosis is confirmed. The latter factor might cause unacceptable delay in the initiation of therapy.

Direct immunofluorescence assay (Integrated Diagnostics, Calif.). Available for more than 2 years as a result of the development of monoclonal antibodies, direct fluorescent antibody staining (DFA) has been found to be more sensitive than the wet mount (80% to 90%), although less so than culture.9, 10 In addition to sensitivity, its advantages include more rapid diagnosis than culture (usually ≤1 day) and stability for up to 7 days, which allows for transportation to a laboratory. Among its disadvantages are the requirement for a fluorescent microscope and a trained microscopist, greater expense than that for culture, and the occasional false-positive result (specificity rate, 99% to 99.5%). Since DFA is a relatively new technique, sensitivity and specificity data are available only from research settings where trained technicians are performing this test. Whether DFA will be as accurate when it becomes translated to the diagnostic marketplace for volume testing cannot be ensured.

Direct enzyme immunoassay. Like the DFA, the monoclonal-based enzyme-linked immunoassay (EIA) offers the advantages of transportability along with sample stability and a greater sensitivity than for the wet mount.11, 12 Similar to DFA, these sensitivity data are generated from research settings and remain to be defined in the clinical or commercial laboratory setting. One major advantage of EIA over DFA is its promise of becoming an in-office procedure that can possibly replace the wet preparation, since only a light microscope is required for interpretation. EIA selectively stains the organism on the basis of monoclonal antibody tagging of the outer membrane protein; however, this ability is somewhat limited in certain geographic areas because of the distribution of aberrant T. vaginalis strains that do not tag readily with the antibody. Because it is a nonamplified assay, the sensitivity of EIA is less than that of culture. Other unanswered questions about EIA are the eventual cost in the clinical setting and the level of expertise required by personnel for accurate results.

Latex agglutination test (Mercia Diagnostics, Ltd., Guildford, Surrey, United Kingdom). Similar to the DFA, this test has a higher sensitivity (95%) than the wet preparation.13 This 5-minute test was basically designed to replace the wet preparation as an easy-toperform test providing immediate results. Cotton swabs containing vaginal secretion are eluted by immersion and agitation in 500 ml glycine-buffered saline. The test is performed by mixing 30 µl eluate with 30 µl of the test and control latex preparations. The mixtures are read on a black slide and checked for agglutination over a 2-minute period. In addition, batch runs are also possible because testing can be delayed. Remaining unanswered questions about this test concern its sensitivity and specificity in the clinical setting and its cost. In a preliminary study of this test by Joseph Lossick, a positive endpoint was difficult to define, with the agglutination often being equivocal. Moreover, at least one batch of reagent supplied by the company produced significant amounts of nonspecific agglutination. With further modifications, the latex agglutination test holds some diagnostic promise for the future. Currently, this test is not commercially available in the United

In summary, these new technologies promise improved accuracy for the diagnosis of trichomoniasis, but they are not likely to replace the wet preparation in gynecologic practice, since it is still a reasonably accurate, simple, and inexpensive procedure. Before physicians abandon the wet preparation, further refinements are needed for some of these assays and the following four issues need to be addressed more fully: (1) definition of the sensitivity and specificity of all new

and traditional diagnostic methods in the clinical arena; (2) quality assurance of clinical and commercial laboratory performance; (3) diagnostic reliability in male patients; and (4) cost-effectiveness of each new diagnostic procedure.

Diagnostic axioms. Inflammatory cells found in the cervical or vaginal secretions should prompt a search for trichomoniasis and other sexually transmitted disease, such as chlamydia, gonorrhea, or herpes. Often, a physician can fine-tune his or her skills in diagnosing *Trichomonas*-based vaginitis by the color of the vaginal epithelium when backup wet preparations and cultures are used as learning tools.

When inflammatory vaginitis exists in the absence of a positive wet preparation, a more sensitive diagnostic procedure such as culture should be used. Because patients without symptoms usually have a low level of *Trichomonas* in the inoculum, other tests, such as DFA and EIA, will probably have a lower sensitivity level than in patients with a positive wet preparation. Culture, on the other hand, should identify the organism if between 1 to 10 are present in the initial specimen.

Incidental Papanicolaou smear findings consistent with trichomoniasis must always be confirmed with wet preparation or other diagnostic procedures if the likelihood of infection is low or unknown, in any given patient.

Treatment of trichomoniasis

Local preparations. Before the 1960s, when systemic metronidazole was first introduced, trichomoniasis generally persisted throughout reproductive life because of the multifocal nature of the disease. Available treatments at the time were local and primarily palliative, offering some symptom relief but only rare cure. Among local medications used were surfacants, pH-lowering agents, and trichofuron. Although therapeutic efficacy was never well defined, available data suggest success in only about 20% to 40% of cases.

Surfacants were designed to "explode" the organisms by creating dramatic osmotic pressure changes that altered the cellular membrane. Also, pH-lowering agents were used in an attempt to make the vaginal environment inhospitable to *T. vaginalis* growth. Clinical cure rates for surfacants and pH-lowering agents ranged from 20% to 40%, but the accuracy of these reported rates is questionable because cultures were used only in the laboratory setting.

Trichofuron, in the form of suppositories or powder insufflations, was used up until the 1960s. Daily powder insufflations were messy for both the patient and the practitioner, cure rates were poorly defined, and patient compliance was minimal.

More recently available local agents include clotrimazole, povidone-iodine, and nonoxynol-9. Clotrimazole was recommended by the CDC as an alternative treatment to metronidazole only a few years ago, but unfortunately, this recommendation was based on three poorly designed studies that claimed cure rates between 60% and 85%. However, broad clinical use after the CDC recommendation revealed symptom relief only, without definitive cure. Likewise, povidone-iodine, which is currently recommended as an alternative treatment in patients for whom metronidazole is contraindicated, produces effective symptom relief but without definitive cure. Nonoxynol-9 shows in vitro efficacy against *T. vaginalis* and also provides symptom relief, but cures appear to be rare.

The nitroimidazoles. The introduction of the nitroimidazoles in 1960 for the systemic treatment of trichomoniasis dramatically changed the therapeutic approach to this infection. Metronidazole is the only nitroimidazole available in the United States, but worldwide the list of similar agents is long—nimorazole, tinidazole, ornidazole, secnidazole, carnidazole, and misonidazole.

Standard treatment with metronidazole is one 250 mg dose, given orally three times a day for 7 consecutive days. Although the efficacy of a single 2 gm oral dose has been confirmed in women,¹⁵ lower efficacy is reported in men.¹⁶ Worldwide, a 1.5 gm dose is usually adequate with more active drugs such as tinidazole. Local treatment with metronidazole suppositories has been tried, but cure rates of only 30% to 60% were reported. This finding is not surprising because trichomoniasis is a multifocal infection involving the Skene's glands, the vaginal epithelium, Bartholin's gland, and the urethra.

Metronidazole resistance. When metronidazole was first introduced, cure rates approximated 95%; but within 2 years of its introduction, the first case of metronidazole resistance was reported in Canada. Nitroimidazole resistance has been reported in most areas of the world and, in recent years, the prevalence of metronidazole resistance seems to be increasing.^{17, 18}

Although methods for determining the sensitivity of *T. vaginalis* are not standardized, the testing procedures developed at the CDC on more than 300 isolates have led to the conclusions that there are four levels of metronidazole resistance in *T. vaginalis* infections—marginal, low, moderate, and high. Moreover, most of the resistant strains have either marginal or very low resistance. Thus metronidazole resistance is not an "all or nothing" phenomenon.

Marginal resistance occurs in one of every 50 to 75 cases, but only one half of these cases will require retreatment. Low and moderate resistance may occur in one out of 200 to 400 cases, and very high levels of resistance occur in approximately one in 2000 to 3000 cases. Fortunately, most cases of nitroimidazole-resis-

tant *T. vaginalis* are readily cured with larger doses of the drug. High-level resistance is, however, very difficult to treat, usually requiring long-term metronidazole therapy with high doses, which may be associated with toxicity.

Treatment regimens that have been found to be curative at different levels of resistance are shown in Table II. Approximately 85% of marginally resistant cases will be cured by retreatment with the standard dose, especially if it is repeated soon after the initial failed treatment, when the organism load is lower. In cases of low-level resistance, easy cure is usually achieved with slightly increased dosage levels. In cases of moderate resistance, a 2.5 gm divided dose for 7 to 10 days will usually cure the infection.

The proper approach to the management of patients with high-level resistance is unclear, since doses of metronidazole required for cure make patients ill. Toxicity includes but is not limited to gastrointestinal upset, metallic taste, glossitis, stomatitis, urticaria, vertigo, and more significantly, convulsive seizures and peripheral neuropathy.

An effective alternative to high-dose oral metronidazole in a highly refractory case was recently reported by Dombrowski et al., who treated a patient with 2 gm of intravenously administered metronidazole every 6 to 8 hours for 3 days. Mild nausea was reported only after the last dose. It is theorized that the side effects of metronidazole are related to the accumulation of the hydroxy metabolite, which occurs around the third or fourth day of treatment—the peak time for most side effects. Although the evidence is anecdotal, neurotoxicity appears to be a consistent problem when high-dose treatment exceeds 3 or 4 days.

Combined oral/vaginal treatment. A recent report of two cases suggests that combination oral/vaginal treatment with metronidazole may be a therapeutic alternative in refractory cases. In this report, 500 mg metronidazole three times a day, in addition to a 1 gm metronidazole suppository three times a day and a 3% acetic acid vaginal lavage two times a week for 2 weeks, successfully eradicated *T. vaginalis* infection: Given the 20% to 30% systemic intravaginal absorption of metronidazole, these cases were probably cured because vaginal treatment contributed to the systemic drug load.

Tinidazole. Although cross-resistance with the nitroimidazoles is the rule, it is often incomplete and many metronidazole-resistant infections resulting from T. vaginalis can be cured with increased but nontoxic doses of tinidazole. In fact, 65% to 70% of T. vaginalis strains highly resistant to metronidazole show increased susceptibility to tinidazole. At the CDC, five documented cases of infection not cured with ≥ 3 gm metronidazole daily for 14 days were cured with tinidazole

Table II. Metronidazole treatment of refractory vaginal trichomoniasis

Resistance level	Metronidazole regimen
Marginal	Retreatment with standard dose
Low	2 gm daily for 3-5 days
Moderate	2-2.5 gm in divided doses daily for 7-10 days
High	3-3.5 gm dailý for 14-18 days
High (alternate therapy)	2 gm intravenously every 6-8 hr for 3 days

at 2 gm daily for 7 to 14 days. Unfortunately, tinidazole, although available in Canada and elsewhere, is not yet available in the United States.

Treatment of trichomoniasis in pregnancy. Because metronidazole crosses the placenta and has been associated with experimental mutagenicity in bacterial systems and carcinogenicity in animals, there has been reticence on the part of clinicans to use metronidazole during pregnancy.²¹ Although data from short-term evaluations suggest that there is no risk of congenital defects when metronidazole is used during pregnancy,²² to date, no study has investigated the oncologic potential of this DNA-acting drug in exposed fetuses. Although one paper suggests the possibility of a metronidazole-induced teratogenic effect,²⁵ this might be coincidental, since other human and animal studies do not support this conclusion.

A recent study suggests that the carriage of *T. vaginalis* is independently associated with low birth weight infants (157 gm or lighter) and premature rupture of the membranes.²¹ This study confirms anecdotal evidence that has linked urogenital trichomoniasis with poor gestational outcomes. However, questions still remain as to whether *T. vaginalis* is the sole etiologic agent associated with premature rupture of membranes or low birth weight, or whether the anaerobic flora of other sexually transmitted diseases that often accompanies trichomoniasis is responsible.

Unfortunately, there are no effective drugs to treat *T. vaginalis* during pregnancy. Treatment of pregnant women who have symptoms might initially consist of 100 mg clotrimazole suppositories daily at bedtime for 2 weeks, which appears to cure about 50% of infections. Caution dictates avoiding use of metronidazole in the first trimester, but a single 2 gm dose may be acceptable in the second or third trimester if prenatal intervention is absolutely necessary and initial treatment attempts fail.

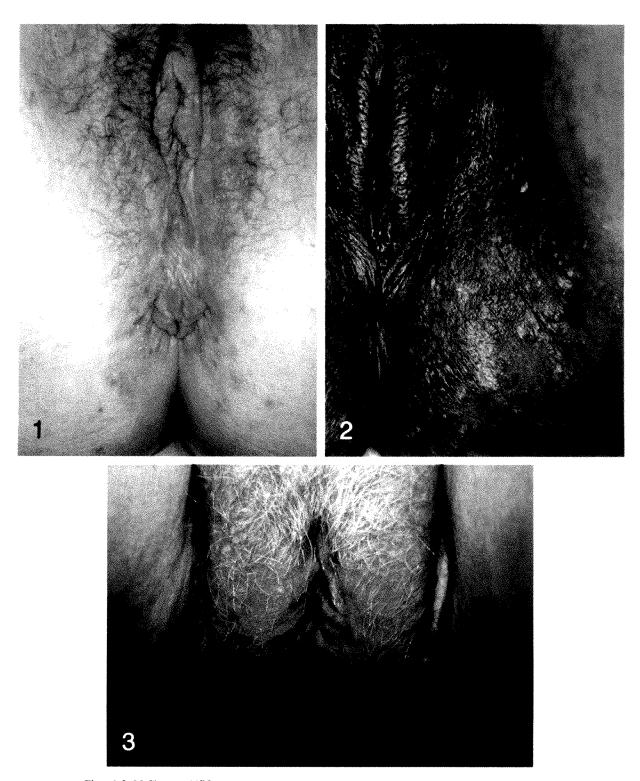
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Color Plates

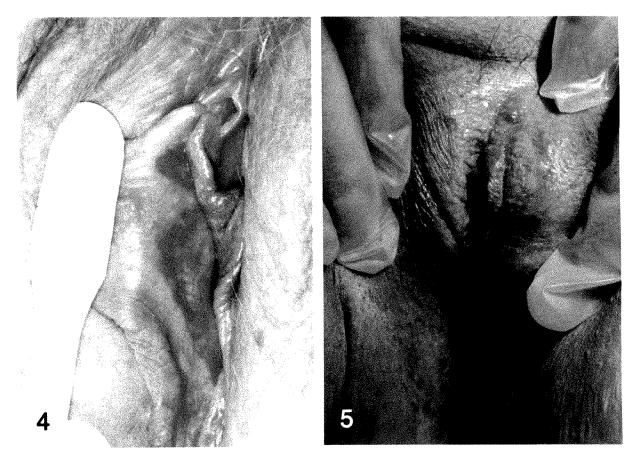


Figs. 1-3, McKay, p. 1176

Fig. 1. Excoriated lesions of lichen simplex chronicus caused by pruritus vulvae and pruritus ani.

Fig. 2. Lichen simplex chronicus in a patient with a long history of tinea infection. Lesions are now potassium hydroxide negative, and thick plaques gradually responded to topical steroids.

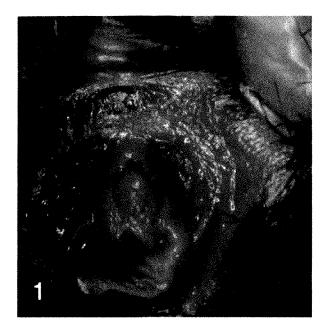
Fig. 3. Rebound erythema of the vulva after withdrawal of a potent topical steroid used for 3 months. The patient complained of burning whenever medication was stopped.



Figs. 4 and 5, McKay, p. 1176

Fig. 4. Erosive vaginitis caused by lichen planus. Red areas have reepithelialized with use of intravaginal and topical desoximetasone cream for 2 weeks.

Fig. 5. Pruritic lichen sclerosus with purpura resulting from patient's scratching. Thickened areas caused by lichenification.





Figs. 1 and 2, Marinoff and Turner, p. 1228

Fig. 1. Diffuse form of vulvar vestibulitis.

Fig. 2. Focal form of vulvar vestibulitis.



Figs. 3 and 5, Marinoff and Turner, p. 1228
Fig. 3. Focal vulvar vestibulitis involving the fourchette.

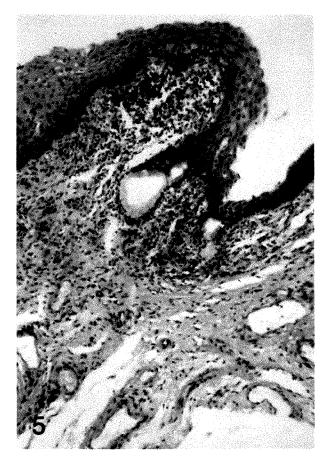


Fig. 5. Chronic inflammatory exudate without gland involvement.

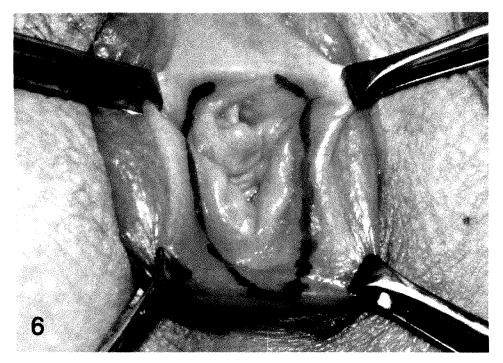


Fig. 6, Marinoff and Turner, p. 1228. Outer incision line for vestibulectomy.

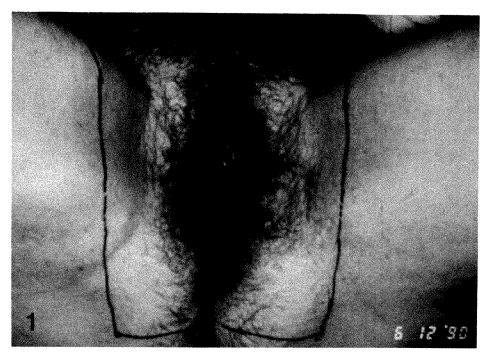
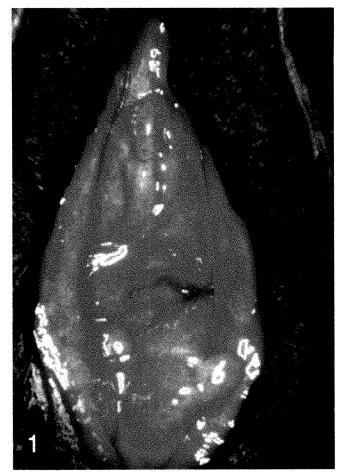


Fig. 1, Turner and Marinoff, p. 1233. Area within the dark line indicates area of altered sensitivity.





Figs. 1 and 2, Wisniewski and Wilkinson, p. 1249.

Fig. 1. Vestibular vaginal red color change in a 4-week puerpera delivered by cesarean section.

Fig. 2. Pallid, friable vaginal color change in a 4-week puerpera delivered vaginally. Mucosanguineous lochial discharge is also present.

Vulvar vestibulitis syndrome: An overview

Stanley C. Marinoff, MD, MPH, and Maria L. C. Turner, MD Washington, D.C.

Vulvar vestibulitis syndrome is a constellation of symptoms and findings involving and limited to the vulvar vestibule that consists of: (1) severe pain on vestibular touch to attempted vaginal entry, (2) tenderness to pressure localized within the vulvar vestibule, and (3) physical findings confined to vulvar erythema of various degrees. Histopathologic findings are consistent with a chronic, nonspecific inflammatory response that is occasionally associated with metaplasia of the minor vestibular glands. The cause is likely multifactorial, and to date the syndrome has been seen in association with subclinical human papillomavirus, chronic recurrent candidiasis, chronic recurrent bacterial vaginosis, chronic alteration of vaginal pH, and the use of chemical and destructive therapeutic agents. Therapy is directed at elimination of these symptoms. When symptoms are unrelieved, a surgical approach consisting of vestibulectomy with vaginal advancement has a high rate of success. (AM J OBSTET GYNECOL 1991;165:1228-33.)

Key words: Vulvar vestibulitis, dyspareunia, human papillomavirus, candidiasis, bacterial vaginosis, vaginal pH

One of the most perplexing problems faced by the practicing gynecologist is that of the patient with chronic vulvar burning and irritation. At the 1983 Congress of the International Society for The Study of Vulvar Disease (ISSVD), the terms "vulvodynia" and the "burning vulva syndrome" were introduced. Vulvodynia was defined as "chronic vulvar discomfort, especially that characterized by the patient's complaint of burning (and sometimes stinging, irritation or rawness)." Further, the ISSVD consensus was that "vulvodynia should be differentiated from pruritus vulvae, which is associated with chronic itching. Vulvodynia can have multiple etiologies, and use of this term for a patient's problem should prompt a thorough diagnostic evaluation." Although vulvodynia by definition means vulvar burning, the spectrum of pain in the vulva area is often described by patients as burning whether elicited by touch or other stimuli. Thorough diagnostic evaluation has led to the establishment of various subsets of vulvodynia, one of which is the vulvar vestibulitis syndrome. This article presents the authors' perspective on this syndrome.

A constellation of symptoms involving and limited to the vulvar vestibule of: (1) severe pain on vestibular touch or attempted vaginal entry, (2) tenderness to pressure localized within the vulvar vestibule, and (3) physical findings confined to vulvar erythema of various degrees has been designated the "vulvar vestibulitis syndrome."²

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The pain may be described as sharp, burning, or a sensation of rawness. Although the sine qua non is introital dyspareunia, the pain may also be elicited on tampon insertion, biking, or wearing tight pants. The present authors first introduced the concept of grading the severity of dyspareunia, which is important in choosing therapeutic options and in evaluating their results. The levels of dyspareunia are a reflection of the patient's subjective perceptions and are described in Table I.

The erythema seen with this syndrome may be diffuse or focal and may be localized around the orifices of the major (Bartholin's, Skene's, or periurethral) or minor vestibular glands or at the fourchette (Figs. 1 through 3, pp. 1224 and 1225).

Historical perspective

Vulvar vestibulitis syndrome has been described by various terminology such as "focal vulvitis" or "vestibular adenitis" for more than 100 years. Skene,3 in his classic Treatise on the Diseases of Women, described a "hyperesthesia" of the vulva that mimics this syndrome. A few years later, Thomas and Munde⁴ described a similar condition., The condition was then forgotten for the next 30 years until 1928, when Kelly⁵ described tender red spots on the vestibule that made intercourse intolerable. In 1942 Hunt⁶ identified the minor vestibular glands but made no connection between them and the syndrome. It was not until 1976 that Pelisse and Hewitt⁷ reintroduced the subject when they described pain with intercourse associated with erythematous vulvitis and plaques in a report of 30 cases. In 1978 Robboy et al.8 described the minor vestibular glands, their origin, and their presence in specimens at autopsy. The association of the female equivalent of plasma cell balanitis of Zoon and a similar entity in women was reviewed by Davis

Table I. Levels of dyspareunia

- Causes discomfort but does not prevent sexual intercourse
- II. Sometimes prevents sexual intercourse
- III. Completely prevents sexual intercourse

et al.9 in 1983. They called the condition "vulvitis circumscripta plasmacellularis" and noted the rarity of this condition. However, their description of the patient's symptoms and the accompanying photograph are classic of the vulvar vestibulitis syndrome. In the same year, Woodruff and Parmley10 reported on 15 patients with dyspareunia and physical findings limited to the vestibule. They first described an operative procedure to correct this problem with satisfactory results in 14 of 15 patients treated. The vulvar vestibule as an entity was described by Friedrich,11 who introduced the term "vestibular adenitis." He concurred about the need for a surgical approach but used the carbon dioxide laser. However, in a later paper, 12 a comparison of the results obtained by different management methods showed that surgery was superior. In 1986 Marinoff and Turner¹³ demonstrated that these patients were not allergic to or irritated by common topical therapeutic agents or their vehicles. In an attempt to consolidate the constellation of symptoms (introital dyspareunia, absence of active infection, erythema around the orifices of the minor vestibular glands, and exquisite tenderness to point palpation) manifested by these patients, the term "minor vestibular gland syndrome" was introduced.

In 1987 Friedrich² reviewed 86 patients with similar signs and symptoms. He introduced the term "vulvar vestibulitis syndrome" and suggested its adoption as the standard description of this disorder. All his patients were white, with a median age of 37 years, although the largest number of patients was between age 20 and 30. Data were retrospectively collected on these patients by a questionnaire. Sixty-three percent of responding patients reported having severe and repeated vaginal candidiasis, and 48% reported that they were "allergic" to one or more substances. Surgery led to significant relief in 60% of patients having surgery.

Except for the statistics on surgical success, these figures mirror the current authors' population. Whether the racial makeup reflects an anatomic variation or a social difference is still to be determined. The difference in surgical success will be discussed in the section on therapy.

Vestibular anatomy

The vestibule is defined as a small cavity or space at the entrance of a canal (Fig. 4). Both Woodruff and Friedrich¹² and Friedrich¹¹ have written review articles on the anatomy of this area. The vulvar vestibule ex-

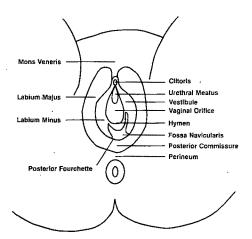


Fig. 4. External genitalia of the female.

tends laterally from the hymenal ring to a line of more keratinized skin on the labia minora, which was first described in 1882 by Hart¹⁴ and is named after him. Hart's line can be visualized in most patients with the naked eye and represents the lateral borders of the vestibule. Anteriorly, the vestibule reaches upward to the frenulum of the clitoris and posteriorly downward to the fourchette. According to Woodruff, "the vestibule represents the only portion of the female genital canal that is of endodermal origin." The urogenital. sinus of the embryo will differentiate into the adult urachus, bladder, urethra, and vestibule. It is covered with nonkeratinized squamous epithelium, an epithelial type that lies someplace between mucous membrane and skin. Within this band of tissue lie the openings of the major (Bartholin's, Skene's, and periurethral) and minor vestibular glands. As an endodermal derivative, it will be less responsive to sex steroids than its surrounding structures, which is something to consider in the treatment of vulvar vestibulitis.

Histopathology of vulvar vestibulitis

The main treatise on this subject was published in 1988. 15 Pyka et al. studied 41 patients who had a vestibulectomy with vaginal advancement performed for the treatment of vulvar vestibulitis syndrome. He demonstrated a mild-to-moderate mixed chronic inflammatory response diffusely distributed in the superficial stroma in all patients. The infiltrate was predominantly characterized by lymphocytes and plasma cells, with only small numbers of polymorphonuclear leukocytes present. When minor vestibular glands were present, they were not affected by the inflammatory cells; thus the term "vestibular adenitis" is a misnomer (Fig. 5, p. 1225).

Superficial mucus-secreting glands were identified in 66% of the patients studied. These minor vestibular glands showed some degree of squamous metaplasia in all patients, some of whom showed complete replaceAm J Obstet Gynecol

Table II. Causes of chronic vulvar vestibulitis

Infections
Subclinical HPV
Bacterial vaginosis
Recurrent candidiasis
Irritants
Chemical therapeutic agents
Destructive therapeutic agents
Altered vaginal pH

HPV, Human papillomavirus.

ment of the columnar epithelium forming what they called a vestibular cleft. Special histologic staining methods failed to demonstrate fungus, gram-positive bacteria, *Mycobacterium* species, spirochetes, or Donovan bodies. No evidence supported an allergic phenomenon or an immediate hypersensitivity reaction, both of which have characteristic histologic pictures. The presence of koilocytosis, histologic suggestion of human papillomavirus infection, was present in 27% of cases.

Turner and Marinoff¹⁶ demonstrated an association between human papillomavirus and the vulvar vestibulitis syndrome. Seven women with signs and symptoms of the syndrome were positive for human papillomavirus deoxyribonucleic acid using Southern blot hybridization. It was suggested that human papillomavirus was one of the causes of the syndrome.

Etiology of vulvar vestibulitis syndrome

Vulvar vestibulitis may be acute or chronic. We use 3 months of symptoms to distinguish between acute and chronic vulvar vestibulitis syndrome. Although this duration may seem arbitrary, it is useful in making therapeutic decisions. We have seen spontaneous regression of symptoms during this time period, especially if a specific cause can be found and treated. Vulvar vestibulitis of any origin invariably causes introital dyspareunia. However, if the introital dyspareunia lasts more than 3 months and becomes the primary complaint, the syndrome must then be classified as chronic.

The causes of vulvar vestibulitis are most likely multifactorial. In the acute form, the rapid relief that is seen when the presumed cause is treated suggests an etiologic relationship. In the chronic form, only an association rather than a direct cause-and-effect relationship can be implied. Many of the causes will be the same for each category, becoming chronic when the cause becomes persistent or recurrent.

Acute etiologies

Infections. Acute vestibulitis may result from any infectious process that causes vulvovaginitis. Candida, Trichomonas, and bacterial vaginosis are examples.

Irritants. Soaps, douches, and sprays are etiologic agents.¹⁷ Of special interest are those patients who have

a tendency to overcleanse with these agents, leading to irritation and symptoms.

Chemical therapeutic agents. Antiseptics, suppositories, or creams, and short-term 5-fluorouracil therapy used as treatment for condylomata acuminata play an etiologic role.

Destructive therapeutic agents. Various treatments, especially those associated with the treatment of condylomata acuminata, have led to the symptom complex. Cryosurgery, trichloroacetic acid, podophyllin, and laser treatment have been implicated.

Drug reactions. Fixed or generalized drug reactions can be seen as etiologic causes.

Chronic etiologies (Table II)

Infections

SUBCLINICAL HUMAN PAPILLOMAVIRUS. Although warty condylomata acuminata rarely cause these symptoms, subclinical human papillomavirus infection identified by a biopsy specimen or hybridization techniques is associated with the syndrome. Squamous papillae of the vulvar vestibule are seen in patients without symptoms and may be just a variation of normal anatomy. In patients with symptoms they tend to be associated with chronic irritation or human papillomavirus. The application of acetic acid and magnification, although nonspecific, will help identify the areas to be biopsied.

Growdon et al.¹⁹ reviewed 12 cases of patients with symptoms with vulvar squamous papillae. Immunoperoxidase staining revealed capsid antigen in two patients, and four of six partners had penile changes consistent with human papillomavirus. They concluded that sexually transmitted human papillomavirus was responsible for the symptoms of vulvar squamous papillomatosis. di Paola and Rueda²⁰ showed similar findings in four patients with vulvodynia and dyspareunia.

We¹⁶ presented seven women with the signs and symptoms of the syndrome who were found by deoxyribonucleic acid hybridization techniques to harbor human papillomavirus. Histologic examination was equivocal in four of seven patients. The clinical presentation before application of acetic acid was not always classic. Smooth epithelial surfaces devoid of papillations and patchy papillations, as well as the more classic florid fine papillomatosis, were seen. We proposed that human papillomavirus infection may be one of the causes of vulvodynia and the vulvar vestibulitis syndrome.

RECURRENT BACTERIAL VAGINOSIS. Alkalinity has been found to be as irritating to the vestibule as acidity. The constant bathing of the vestibule with the alkaline discharge of chronic bacterial vaginosis can lead to symptoms of vulvar vestibulitis syndrome. Treating the infection with the subsequent removal of the irritating discharge can induce amelioration of symptoms.

CHRONIC CANDIDIASIS. This term refers to chronic,

recurrent, culture-positive disease. Many patients state they have recurrent "yeast" infections when in reality they have other problems.21 Recurrent Candida vaginitis is defined by Sobel²² as the occurrence of at least four documented episodes in a 12-month period. It is often associated with chronic vaginal soreness and dyspareunia.23 We13 were unable to prove that persistent hypersensitivity to previous Candida infection was the cause of the syndrome. Also, it was neither caused by irritation nor allergic reactions to treatment vehicles. Ashman and Ott24 proposed autoimmunity as a factor in recurrent vaginal candidiasis and vulvar vestibulitis and presented an animal model. Although many patients have a history of prior "yeast" infections, the cause-and-effect relationship has not been proved.

Altered vaginal acid-base balance. As with bacterial vaginosis, other conditions that significantly alter vaginal pH can lead to chronic irritation and symptoms. Estrogen-deficient states, severe cervicitis, and the decrease in or absence of lactobacilli have been implicated.25

Miscellaneous. Other associated conditions include previous systemic chemotherapy, association with lichen sclerosus, inflammatory bowel disease, and interstitial cystitis. The mechanism of these relationships is still to be determined.

Treatment

The treatment for acute vulvar vestibulitis syndrome is medical. First, any specific infection found should receive pharmacologic treatment. Second, all therapeutic modalities, local or systemic, that may contribute to the problem should be discontinued. Finally, topical steroids should be started when the infection is controlled. Surgery is not indicated.

The treatment for chronic vulvar vestibulitis syndrome is much more complicated. Some success has been obtained when a specific etiologic condition can be identified and treated. Even here, treatment of the underlying cause does not yield a cure in many cases. Surgery has become the treatment of choice when all other modalities fail.

Subclinical human papillomavirus. With the advent of approval by the Food and Drug Administration of recombinant interferon α-2B for the treatment of condylomata acuminata, the use of this treatment for patients with subclinical human papillomavirus vulvitis and symptoms of vulvar vestibulitis syndrome became available. Hatch26 treated 22 women who had subclinical human papillomavirus infection (as proved by colposcopic examination, biopsy, and deoxyribonucleic acid hybridization) with interferon α-2B injected directly into the vulva with good results in 16 of 20 patients. He did not specifically comment on the relief of symptoms but relied on colposcopic examination and

human papillomavirus deoxyribonucleic acid probe analysis to evaluate the response. Horowitz²⁷ specifically limited his study to patients with vulvar pain characteristic of the vulvar vestibulitis syndrome and pathologic biopsy showing evidence of papillomavirus. All were at level III dyspareunia. Fifteen of 17 women responded favorably, with total absence of vulvar pain. Kent²⁸ repeated the same study in five patients, with a successful outcome in four of five patients. Of the 29 patients treated in the same fashion by the present authors, 13 had complete remission of symptoms, 3 had some improvement, and 13 had no improvement. In the same study, a cost-benefit analysis comparing interferon injections and a surgical approach showed that significant savings were realized by first treating patients with interferon rather than by immediate surgery.

Recurrent bacterial vaginosis and altered vaginal pH. The authors have identified a group of patients who have in common elevated pH of the vaginal secretions. These women complain of a copious discharge as well as the classic symptoms of vulvar vestibulitis. A distinctive observation of patients is that the symptoms worsen with an increase in the amount of the discharge. When a specific cause can be found and eliminated, there is marked improvement in their symptoms.

Recurrent bacterial vaginosis is a disease of unknown origin that results from massive overgrowth of vaginal bacterial flora, resulting in an alkaline discharge at the introitus.29 Treatment of this disorder with metronidazole or clindamycin has led to improvement in the symptoms of vulvar vestibulitis. Estrogen-deficient states may lead to secondary infection with various organisms, especially β-streptococcus. Chronic cervicitis can produce a constant discharge that tends to have an alkaline pH. Some patients have a lack of Lactobacillus not in association with bacterial vaginosis. Treating the primary condition with antibiotics, cryosurgery, or repopulating the vagina with Lactobacillus and reducing the alkalinity has been successful in improving the symptoms of vulvar vestibulitis. Care should be taken to choose a hydrogen peroxide-producing Lactobacillus product for this treatment.30

Chronic recurrent candidiasis. The association between chronic recurrent candidiasis and symptoms of the vulvar vestibulitis syndrome has been discussed. Various treatment regimens have been used with varying success. When this diagnosis can be verified, longterm treatment with oral ketoconazole has led to an improvement in the symptoms of the vulvar vestibulitis syndrome.

Surgical approach

Surgery should be reserved for patients with level II or III dyspareunia of at least 6 months' duration who

Table III. Results of surgical treatment of vulvar vestibulitis syndrome

Results	No. of	patients (%)
No improvement	2	(3%)
Partial improvement	11	(15%)
Complete cure	60	(82%)
Total number of patients	73	, ,

have not responded to treatment for a specific cause or for whom no cause can be established.

Woodruff et al.31 first described a procedure for the treatment of dyspareunia and vaginal outlet distortions. Eighteen of 42 patients had dyspareunia as the primary symptom; four were at level III. Dyspareunia was alleviated in all patients by a combination of surgery and, in appropriate cases, adjunct therapy for other causes. In 1983 Woodruff and Parmley¹⁰ described 14 patients with dyspareunia and minimal physical findings except for tender red papulés in the vestibule who underwent a procedure similar to that described previously. All these patients achieved major relief of symptoms. The incision extended from about 0.5 cm beneath and lateral to the urethra to the fourchette, and the vagina was mobilized to cover the defect. Between 1982 and 1984, 64 patients underwent this procedure with good results in 80% of the cases.32 It was recognized that the minor vestibular glands extend lateral to the urethra and on occasion above the urethra. Involvement of the opening of the Bartholin duct was described by Michlewitz et al.,35 who demonstrated that laser vaporization was not as effective as surgery in the treatment of these patients. This had been recognized previously by Woodruff and Friedrich.¹² When Friedrich² reviewed his patients in 1987, he found that surgery produced only a 60% cure rate. He used a similar technique as described in Woodruff and Friedrich's earlier articles. Similar results were obtained by other surgeons.34 With modification of this technique to include the periurethral areas, we and others have been able to obtain a 90% to 95% improvement rate in selected patients who meet the discussed criteria for surgery (Table III). The outer incision line extends from the periurethral glands on one side, along Hart's line, down into and including a good portion of the fourchette and back along Hart's line to the periurethral glands on the other side (Fig. 6, p. 1226). The inner incision line is behind the hymenal ring. The horseshoe-shaped tissue in between these lines is then completely excised, and the vaginal mucosa is mobilized and advanced to cover the defect. Complications include wound hematoma, partial or complete dehiscence, uneven healing requiring minor revision, and Bartholin's duct stenosis with cyst formation. Recent attempts at combining laser vaporization and vaginal advancement have not seemed to improve the results obtained with surgery alone.35

Conclusions

Too often patients with vulvar symptoms are shunted from one gynecologist to another and finally told they should seek psychiatric help. Although psychotherapy is an often neglected but very important aspect of total patient management, when there is a definite constellation of signs and symptoms, every effort should be made to elicit the cause and available treatment initiated. Vulvar vestibulitis is a syndrome with a definite set of signs and symptoms, predictable pathology, and sometimes known etiology. When a specific cause can be found, treatment yields relatively good success. Even in idiopathic cases, nonspecific treatment can sometimes be helpful. In recalcitrant cases, surgery is the only other means of obtaining relief for these patients.

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Pudendal neuralgia

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We call attention to a group of patients with chronic vulvar burning (vulvodynia), who do not have apparent infections or easily discernible abnormal physical findings, but who on simple sensory testing have allodynia, hyperalgesia, hyperpathia, and hypoesthesia in varying permutations within the areas innervated by the pudendal nerve. We propose that pudendal neuralgia (pain along the pudendal nerve) is one of the causes of idiopathic vulvodynia. In those patients in whom a neurologic, metabolic, infectious, traumatic, or malignant cause for the neuralgia is not found, medical management with tricyclic antidepressants, antiepileptic agents, or both may prove helpful. Awareness of this entity will lead to earlier diagnosis, treatment, and reassurance of patients with chronic vulvar burning. (AM J OBSTET GYNECOL 1991;165:1233-6.)

Key words: Pudendal neuralgia, vulvodynia, allodynia, herpes simplex, tricyclic antidepressants

Interest and more directed study of the possible causes of chronic vulvar pain did not start until the early 1980s when the International Society for the

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Study of Vulvar Disease set up a task force on the "burning vulva syndrome." In 1983 this task force applied the term "vulvodynia" to chronic vulvar discomfort characterized by the patient's complaint of burning, irritation, or rawness. Although there had been sporadic reports in the literature and short entries in text-books on some pathologic causes of chronic vulvar burning and discomfort, the psychosomatic origins of these complaints received as much if not more atten-

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tion.^{2,3} In a state-of-the-art paper, McKay⁴ summarized the currently known causes or conditions associated with vulvodynia: vulvar dermatoses, recurrent candidiasis, vulvar vestibulitis, and a group labeled "essential vulvodynia." This last group of patients said to have "essential vulvodynia," who complain of constant or nearly constant vulvar burning and have a paucity of physical findings, will be the focus of this article. We propose that most of these patients suffer from pudendal neuralgia. The possible causes of pudendal neuralgia are varied, and we include citations from the literature and the results of our investigations.

Definition and history

As an entity, pudendal neuralgia has not merited much attention in textbooks of neurology. Wilson⁵ described this condition as characterized by "mostly vague but sometimes well-defined pains, referred to perineum, scrotum, testis, penis, and spermatic cord...often very severe and at times paroxysmal." He described extreme sensitivity to touch such that even light pressure became intolerable. Men appeared to be more commonly afflicted, although women could be similarly afflicted in analogous locations. In his experience, the majority of such patients were neurotic. Earlier Zuelzer6 called attention to the fact that irritation of the pudendal nerve could be mistaken for cystitis due to the complaints of frequency and dysuria. However, the accompanying increase in skin sensitivity in the perineum made him recognize these cases as pudendal neuralgia.

Anatomy

The perineum has an overlapping nerve supply⁷ with some branches from the iliohypogastric nerve (T-12 and L-1), the ilioinguinal nerve (L-1), and the genitofemoral nerve (L-1 and L-2). However, the vast majority of its nerve supply (sensory, motor, and autonomic) is drawn from the pudendal nerve (S-2, S-3, and S-4), which eventually divides into the perineal nerve, the dorsal nerve of the penis or clitoris, and the inferior hemorrhoidal nerve. Using a needle for testing, Zuelzer was able to delineate a rhomboidal area of increased sensitivity around the perineum. The anterior border was a few centimeters above the symphysis pubis, the posterior border was behind the anus, and the upper, inner thighs formed the lateral limits (Fig. 1, p. 1226).

Symptoms

Almost all patients complain of an unprovoked, persistent, superficial, burning sensation that is frequently accompanied by a deep, aching component. Some patients also experience rare, paroxysmal lancinating or stabbing pains over a larger area. Patients who are less severely involved initially complain of an itch-burn sen-

sation or a feeling of rawness. Some patients perceive burning pain when the vulvae are lightly stroked with a cotton wisp (allodynia). This corresponds with their frequent observations that light touch, clothing, water, and even the motion of their pubic hairs lead to burning pain. It follows that these patients complain of dyspareunia not only on penile penetration but also during and after intercourse. Patients who are more severely affected are unable to have intercourse. It is not unusual for patients to report rare, unexplained, symptom-free periods lasting for days to weeks.

The above set of symptoms are felt over various areas supplied by the branches of the pudendal nerve, unilaterally, bilaterally, or in an asymmetric fashion. The following structures are seen to be involved singly or in various combinations: cutaneous surfaces of the labia majora and minora, clitoris, urethral meatus, vulvar vestibule, anus, perineum, and perianal skin.

Involvement of the urethral meatus leads to "cystitis-like" symptoms of frequency and dysuria in the presence of negative cultures. "Sciatica-like" pains are sometimes reported.

Physical findings

There is usually a paucity of physical findings. The vulvar lining may have variable textures. Some appear to have normal markings, whereas others seem smooth and nearly sclerotic. Some labia appear somewhat hypertrophic and cobblestoned. There is generally no evidence of infection, abnormal wet smears, or pH changes. This may explain why women who have the above constellation of symptoms are frequently labeled as hysterical, neurotic, or malingering.

Focal vestibular erythema with or without tenderness is sometimes seen. The relationship of these findings to this entity is not clear.

Positive findings that may be helpful include the presence of thickened surgical scars at the painful site, the presence of a palpable tumor, and evidence of genital herpes. It is sometimes helpful to examine the patient at the height of the symptoms, because this might be an opportune time to see herpetic vesicles. This has been our experience innumerable times.

Sensory testing with a cotton wisp and a sharp pin may elicit any combination of the following responses:

Allodynia: Pain caused by a stimulus that does not normally provoke pain.

Hyperalgesia: An increased response to a stimulus that is normally painful. An example is severe sharp pain provoked by a light pin-prick.

Hyperpathia: A painful syndrome characterized by increased reaction to a stimulus, especially a repetitive stimulus. Delay in sensation, radiating sensation, and af-

ter sensation are other qualities that define this condition.

Hypoesthesia: Diminished sensitivity to sensory stimulation.

Diagnostic tests

Among useful diagnostic assays are the Tzanck smear and culture for herpes simplex from suspicious lesions anywhere along the lower genital tract. Determination of evoked potentials can also be obtained, but this is not an easy test to perform in this area, and norms are not clearly established. Medical examination and blood chemistries should be obtained when indicated to rule out metabolic causes of neuropathy. Radiographic studies of the lumbosacral spine may be performed to rule out disk problems, arthritis, or space-occupying lesion.

Etiology

Pudendal neuralgia has been reported after surgical and nonsurgical trauma,8 sports trauma such as "unicyclist's sciatica,"9 and from horseback riding.10 It has been seen in association with a solitary neurofibroma¹¹ and in one of our patients with multiple sclerosis. The most common association, as seen in our practice and as reported in the literature, is with herpes simplex.12, 13

Treatment

Depending on the severity of the problem, a combination of several modalities may be necessary.

- 1. Nonsteroidal antiinflammatory agents, although frequently used, have not proved helpful in most cases.
- 2. In mild cases, a tricyclic antidepressant such as amitriptyline^{14, 15} at a dose of 10 to 75 mg before bedtime is helpful. It may take 2 to 3 weeks to reach efficacy, and its use may be limited by side effects that include sedation, excitation, and anticholinergic effects such as dry eyes and mouth, weight gain, and photosensitivity. Imipramine and other tricyclics may be substituted for amitriptyline until a tolerable one is found. The analgesic effects occur at doses much lower than those required for antidepressant effects.
- 3. Anticonvulsants, such as phenytoin and carbamazepine,16 have been used successfully in postherpes zoster neuralgia. Carbamazepine may be started at a dose of 100 mg, increased by increments of 100 mg every 2 days until a daily dose of 600 mg is reached unless pain relief occurs at a lower dose. It should be administered every 8 hours to maintain therapeutic blood levels. Because of hematologic, dermatologic, and hepatic toxicities, close monitoring is necessary. Phenytoin

- is given at the rate of 300 to 400 mg a day in two divided doses to attain adequate serum levels. If no relief of pain is achieved in 3 weeks, the drug should be discontinued, because higher doses lead to toxicity.
- 4. Systemic acyclovir, 200 mg three times daily, for long-term prophylaxis in proven cases of recurrent genital herpes simplex is helpful to prevent the acute pain associated with recurrence. Alone it does not alleviate the chronic burning component.
- 5. Surgical procedures, such as nerve sections, excision of scar tissue, sympathectomy by way of dorsal root entry zone, may be tried in recalcitrant cases.
- 6. Miscellaneous therapies, such as acupuncture, transcutaneous electrical stimulation, topical anesthetics, regional blocks, and capsaicin, have been tried with inconsistent results. Although capsaicin is considered helpful for postherpes zoster neuralgia, the few patients we have treated could not bear the vulvar stinging from its use.
- 7. Psychotherapy is an often neglected but very important aspect of total patient management. Studies have shown a very high incidence of depression in patients with long-term pain of more than 3 months' duration.

Conclusion

We have attempted to call the attention of practicing gynecologists, dermatologists, and other physicians who treat women with vulvar problems to another possible cause of chronic vulvar burning. It is particularly useful to consider this diagnosis when there are few physical findings. A screening sensory evaluation will quickly determine whether pudendal neuralgia is a serious consideration and whether referral to a neurologist might be indicated. Our current experience with therapy has not been encouraging except for the milder cases or those that are not chronic. More research is necessary to determine optimal treatment.

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Toxic shock syndrome: An update

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Since late 1979 to early 1980, when toxic shock syndrome achieved notoriety, a substantial body of data has demonstrated that vaginal Staphylococcus aureus infections, particularly those occurring during menstruation, account for most cases of toxic shock syndrome in women of reproductive age. Among those patients with onset during menstruation, tampon use has been identified as the most important risk factor. Although menstrually associated cases have been observed among users of all brands and styles of tampons, the use of selected "super absorbent" brands and styles has been associated with an increased risk of toxic shock syndrome. Although the tampon characteristics responsible for the associated risk of toxic shock syndrome remain poorly understood, both absorbency and chemical composition appear to be important variables in this relationship. In response to studies demonstrating this relationship and legal pressures, tampon manufacturers have removed chemical constituents previously used to enhance absorbency, such as polyester foam, carboxymethylcellulose, and polyacrylate rayon, and have markedly reduced tampon absorbency. Coinciding with these changes, the reported number of menstrually related cases of toxic shock syndrome has dropped substantially, although they continue to account for 50% to 70% of all cases of toxic shock syndrome in women of reproductive age. (AM J OBSTET GYNECOL 1991;165:1236-9.)

Key words: Toxic shock syndrome, tampons, Staphylococcus aureus

Toxic shock syndrome (TSS) is a severe, potentially life-threatening systemic illness resulting from infection with toxin-producing strains of Staphylococcus aureus at any body site. TSS achieved notoriety in 1980, when large numbers of cases among young, previously healthy women who were menstruating began to be noticed by clinicians and public health officials. Since then, much has been learned about the clinical and epidemiologic aspects of TSS, and in vivo and in vitro laboratory studies have elucidated the bacterial prod**Table I.** Simplified case definition of TSS

Fever: Temperature ≥38.9 °C (102° F) Rash: Diffuse macular erythroderma Hypotension: Systolic blood pressure <90 mm Hg or orthostatic changes in blood pressure Desquamation 1-2 wk after onset of illness Evidence of involvement of ≥3 body systems (gastrointestinal, muscular, mucous membrane, renal, hepatic, hematologic, and central nervous system) No evidence of another cause for the illness

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ucts and the elements of the host response that combine to produce the pathophysiologic changes seen. Based on the results of the epidemiologic and laboratory studies, as well as on legal and financial considerations, the manufacturers of tampons have dramatically altered the products they market. The incidence of tampon-

Table II. Complications of TSS

Acute renal failure Adult respiratory distress syndrome Metabolic acidosis Electrolyte disturbances (hypocalcemia, hyposphatemia, hypomagnesemia) Disseminated intravascular coagulation Encephalopathy Cardiomyopathy Hair and nail loss

associated TSS has declined substantially since 1980 at least partially because of such changes.

Clinical features

For epidemiologic purposes, a strict case definition was developed for TSS in 1980,1 a simplified version of which is presented in Table I. Although epidemiologists and (to an even greater extent) lawyers have relied on this case definition to divide the world neatly into those patients who do and those who do not have TSS, most clinicians recognize that many patients with what undoubtedly are milder cases of TSS do not fulfill all the listed criteria. Although virtually all patients with TSS severe enough to meet this case definition require hospitalization at least for a few days, it is likely that many milder cases of TSS are treated on an outpatient basis or resolve spontaneously. Although unproved, it seems reasonable to suspect that in mild cases the amount of toxin released is reduced or that such cases occur in persons who are partially immune because of prior exposure to the responsible toxin. It is also possible that variation in the severity of cases is due to differences in the infecting S. aureus strains, either in the amounts or the type of toxin being produced and released.

Currently TSS meeting the strict case criteria has a case-fatality rate of approximately 2%, although fatality rates of 4% to 5% were noted in the early 1980s. Although a wide range of complications has been observed in severe cases of TSS (Table II), adult respiratory distress syndrome, acute renal failure, and disseminated intravascular coagulation have been the most troublesome. A number of characteristic laboratory changes also are seen in TSS (Table III), although none is unique to TSS.

The treatment of TSS remains largely supportive. Initially the most important are adequate drainage of any infected site to further minimize uptake of toxin; replenishment of intravascular volume with sometimes prodigious amounts of replacement fluids; support of blood pressure with pressor agents when required; and treatment of life-threatening complications such as respiratory or renal failure. Treatment with an antimicrobial agent effective against S. aureus is also a routine part of the management of TSS, although it may be

Table III. Distinctive laboratory changes in TSS

Immature and mature neutrophils >90% of white blood cell differential Pvuria Hypocalcemia Hypophosphatemia Hypoferrinemia Hypoproteinemia/hypoalbuminemia Azotemia Increased liver enzyme Increased creatine phosphokinase Isolation of S. aureus from infected site

more important in preventing recurrences than in improving outcome of the acute illness. High-dose corticosteroids, although reported to be of some benefit in severe TSS, have not been well studied. Treatment with human immune serum globulin, which can be expected to contain antibodies to the toxin responsible for most cases of TSS, might theoretically be of benefit in treating TSS by binding circulating toxin, although there is no clinical experience with such therapy.

Descriptive epidemiology

Since 1980, there has been a dramatic decline in the number of cases of TSS reported through the national passive surveillance system (Fig. 1).2 Some of the increase in TSS cases seen in 1980 and some of the subsequent decrease are undoubtedly due to artifacts produced by changes in disease recognition and reporting. However, it is interesting to note that reporting of nonmenstrual cases of TSS does not appear to have been affected by such artifacts, with a relatively constant number of such cases reported annually for the past 10 years. Furthermore, evidence from other studies^{3, 4} in which artifacts were eliminated show similar trends in the incidence of menstrual TSS. These trends are quite consistent with and largely explained by documented changes in the chemical composition, absorbency, and usage patterns of tampons during the same time period.

The current distribution of TSS cases is best reflected in data collected using active, hospital-based surveillance in five states and one large county in 1986 and 1987.5 Of the 179 cases of TSS detected by this active surveillance system, 85% were in girls and women and 15% were in boys and men (Table IV). Of the 152 cases in girls and women, only 55% had onset during menstruation, whereas another 10% were associated with use of barrier contraception and 7% occurred in postpartum women. The remaining cases in men and women were due to S. aureus infections at a wide array of body sites. Data from the same study, as well as data from a chart review study designed to find all cases of TSS in men and women 15 to 34 years of age whether

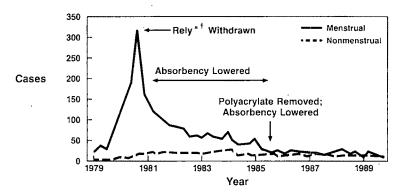


Fig. 1. Reported cases of TSS (includes only cases meeting the case definition of the Centers for Disease Control) by quarter. United States, Jan. 1, 1979 to March 31, 1990. (From Centers for Disease Control. Reduced incidence of menstrual toxic shock syndrome—United States, 1980-1990. MMWR 1990;39:421-3.)

Table IV. Distribution of conditions associated with TSS by gender of patient

	No. of cases (% of total)				
Case type	Female	Male	Total		
Menstrual*	83 (54.6)		83 (46.4)		
Barrier contraceptive†	15 (9.9)		15 (8.4)		
Postpartum‡	10 (6.6)	· ·	10 (5.6)		
Nonsurgical wound	10 (6.6)	16 (59.3)	26 (14.5)		
Surgical wound	11 (7.2)	4 (14.8)	15 (8.4)		
Other, unknown	23 (15.1)	7 (25.9)	30 (16.8)		
TOTAL	152 (84.9)	27 (15.1)	179 (100)		

From Gaventa S, Reingold AL, Hightower AW, et al. Active surveillance for toxic shock syndrome in the United States, 1986. Rev Infect Dis 1989; II:S28-S34. Reproduced with permission of Infectious Diseases Society of America, and with permission of the publisher, The University of Chicago Press.

*Onset of illness during or <24 hours after menstrual bleeding.

†Contraceptive sponge, diaphragm, or cervical cap in place at onset of illness.

‡Onset within 30 days after termination of pregnancy (i.e., postpartum or after miscarriage or abortion).

diagnosed or not,⁴ document that young women (15 to 19 years) are at greater risk than older women of menstrual age and that white women are at greater risk than nonwhite women. Previously observed variations in the incidence of menstrual TSS in different regions of the United States appear to have largely disappeared, and the current incidence of menstrual TSS in all regions from which there are appropriate data is in the range of 1 to 3/100,000.

Risk factors for TSS

Numerous studies consistently and convincingly have shown that tampon use is the major risk factor for TSS occurring during menstruation. Even in 1986 to 1987, after enormous changes in the chemical composition of tampons and marked reductions in their absorbency, an elevated risk of menstrual TSS among tampon users was redemonstrated. Epidemiologic studies from the early and mid-1980s demonstrated that the risk of menstrual TSS varied directly with the absorbency of the tampon used and suggested that the chemical com-

position of the tampon also was important in determining the risk of TSS.⁷⁻⁹ In vitro studies confirm that the chemical composition of a tampon can markedly affect the production of the staphylococcal toxin thought to be involved in more than 90% of all menstrual cases of TSS.^{10, 11} Among nonmenstrual cases of TSS associated with *S. aureus* infections of the vagina, the use of barrier contraception (diaphragms and contraceptive sponges) is the only known risk factor.¹²

Microbial factors and pathogens

As noted above, TSS can be caused by infections at any body site with strains of *S. aureus* that produce certain exoprotein toxins. The overwhelming majority (>90%) of menstrual TSS cases are caused by *S. aureus* strains that make TSS toxin-1 (TSST-1), the toxin most clearly identified with TSS.¹³ The ability of purified TSST-1 to produce a similar illness in rabbits and the ability of antibody to TSST-1 to block such illness demonstrate convincingly that TSST-1 can produce TSS. However, many (>30%) of the *S. aureus* strains recov-

ered from patients with nonmenstrual TSS do not make TSST-1, and it appears that staphylococcal enterotoxins, particularly enterotoxins B and C, can produce a clinically indistinguishable syndrome.

TSST-1 and the staphylococcal enterotoxins now appear to be extremely interesting molecules that can act as "super antigens," having profound effects on the human immune system and producing an outpouring of various endogenous factors, such as the interleukins and tumor necrosis factor.14 Furthermore, the production of TSST-1 by S. aureus has now been shown to be markedly dependent on the level of ambient oxygen, glucose, magnesium, and other factors, some of which can be introduced into or removed from the vagina by the presence of tampons of various compositions. Thus tampons may increase the risk of menstrual TSS by affecting the level of various factors that in turn affect the production of TSST-1.

Host factors

The presence of toxin-producing S. aureus at a body site, although necessary for TSS to develop, is clearly not sufficient. Numerous studies have demonstrated that nasal and vaginal colonization with TSST-1-producing S. aureus is fairly common, whereas TSS is quite rare. Thus it is clear that other conditions must be met for TSS to develop. One factor likely to be important in determining whether TSS will develop is the presence or absence of preexisting antibody to TSST-1. Although the source remains obscure, it is clear that antibodies to TSST-1 are acquired during the first 15 to 20 years of life, such that by age 20 to 25 years, more than 90% of men and women have detectable anti-TSST-1 antibodies. If, as is believed, such antibodies protect against the development of TSS, then only a small proportion of people are susceptible to developing TSS when infected with a TSST-1-producing strain of S. aureus. However, even after taking such serosusceptibility into account, as well as what proportion of menstruating women use tampons and what proportion are infected with a TSST-1-producing strain of S. aureus, the observed number of menstrual cases of TSS is much lower than would be predicted. Therefore other currently unknown host factors and vaginal conditions must also be important in determining which serosusceptible, menstruating women who are infected with such a strain will develop TSS.

Prevention

Despite the dramatic changes in tampon formulation since the early 1980s and the observed sharp decline in menstrual TSS, the risk of menstrual TSS still appears to be elevated among users of many, if not all, currently available brands and styles of tampons.7 If a

woman wishes to reduce her already very small risk of developing menstrual TSS, she can do so by using napkins or pads instead of tampons. Because this choice will be unacceptable to many women, those women who continue to use tampons can be advised to use the lowest absorbency tampons compatible with their needs and to alternate tampons with pads or napkins, perhaps using the latter at night. Although frequent changing of tampons has never been demonstrated to reduce the risk of menstrual TSS, it is reasonable to suggest that an individual tampon not be left in place too long. Women who use tampons should read the warning label or package insert concerning TSS that is now found on or in all tampon packages. If symptoms compatible with TSS develop, a woman should remove her tampon and seek prompt medical attention.

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Nonbarrier contraceptives and vaginitis and vaginosis

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Within the limitations of the available data, it has been reported that oral contraceptive use versus other contraceptive methods is associated with a greater or similar frequency of candidiasis, increased numbers of anaerobic microorganisms, an increased or similar frequency of *Chlamydia trachomatis*, and a reduced frequency of bacterial vaginosis and trichomoniasis. The impact of contraceptive steroids on cellular and humoral immunologic factors may explain these observations. Intrauterine contraceptive device use is reported to be associated with an increased rate of bacterial vaginosis and anaerobic organism recovery from the vagina regardless of symptoms. Patients having a contraceptive vaginal ring were found to have the same number and types of vaginal organisms as oral contraceptive users. Levonorgestrel-releasing subdermal implant (Norplant, Wyeth-Ayerst, Philadelphia, Pa.) users have been reported to have approximately half the rate of vaginitis and vaginosis compared with that of Copper T-200 intrauterine device users. (AM J OBSTET GYNECOL 1991;165:1240-4.)

Key words: Contraceptives, oral contraceptives, intrauterine devices, vaginitis, vaginosis, candidiasis, *Chlamydia trachomatis*, trichomoniasis

The ideal manner to study the relationship between contraceptive use and the development of vaginitis and vaginosis would require a prospective, randomized study adjusted for age, parity, sexual activity, smoking, and other potentially confounding factors. Studies incorporating all these aspects have not been performed. Typical reports, even if prospective, study nonrandomized allocation of patients to contraceptive methods and are limited in scope. Usually patients who complain of vaginitis or vaginosis have been investigated to assess their use of contraceptives. Case-control studies such as these, although easier to perform than cohort studies, have their usual biases and limitations. Thus only the number of patients with these complaints who seek medical attention (the numerator) is available, and we do not know the number of patients who use the various methods of contraception (the denominator). This type of information does not permit the calculation of predictive value (or likelihood) of the development of vaginitis and vaginosis by use of each method of contraception. Thus we can only report the frequency with which each method of contraception is used by the type of vaginitis and vaginosis being considered.

Material and methods

A literature search was performed. Studies have been included in this review that appeared to provide useful information regarding the association of vaginitis and vaginosis with the use of oral contraceptives (OCs), intrauterine devices (IUDs), contraceptive vaginal ring (a

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vaginal method but not a barrier method), and Nor-plant.

Results

Osborne et al.1 examined the relationship between vaginitis and sexually transmitted organisms in sexually active women. Symptomatic women (n = 253) and a control population of asymptomatic women (n = 130)were identified and examined. Each group used the same proportion of birth control methods (OCs, 64% and 72%; IUDs, 7% and 6%; barrier contraception, 11% and 12%; and other contraception, 18% and 10%, respectively). Therefore the method of birth control was ignored in the remainder of the study. When the potentially sexually transmitted disease organisms recovered from the symptomatic and asymptomatic groups were examined (Table I), herpes simplex, Neisseria gonorrhoeae, Gardnerella vaginalis (a marker for bacterial vaginosis), Trichomonas vaginalis, and yeasts were significantly more prevalent in the symptomatic than in the asymptomatic group. Conversely, as one would expect, the asymptomatic group was more likely to have none of the above. However, the incidence of Chlamydia trachomatis was not significantly different between these two groups, nor was the incidence of genital mycoplasmas or group B streptococcus. Thus from this relatively recent study published in 1982, it would appear that vaginitis is not associated with the method of birth control.

However, if we review earlier literature on candidiasis (Table II), a prevalence study by Jensen et al.² published in 1970 found a 15% rate of candidiasis in OC users compared with a 5% rate in non-OC users—a significant difference. A prevalence study by Catterall³ in 1971 reported that 99 of 200 patients with vaginal

Table I. Prevalence of specific microorganisms in sexually active women

	Symptomatic		Asympto	Asymptomatic [*]		
Microorganism	No.	%	No.	%	Significance* (p value)	
C. trachomatis	24/253	9.5	9/130	6.9	0.1983	
Mycoplasma hominis	65/253	25.7	19/130	14.6	0.0722	
Ureaplasma urealyticum	48/253	19.0	24/130	18.5	0.9834	
G. vaginalis	60/253	23.7	7/130	5.4	0.0001	
N. gonorrhoeae	27/253	10.7	3/130	2.3	0.0001	
Group B streptococci	51/253	20.2	23/130	17.7	0.8580	
Yeasts	99/253	39.1	29/130	22.3	0.0473	
T. vaginalis	62/253	24.5	9/130	6.9	0.0002	
Herpes simplex	32/253	12.6	2/130	1.5	0.0000	
None of the above	23/253	· 9.1	76/130	58.5	0.0001	

From Osborne NG, Grubin L, Pratson L. Vaginitis in sexually active women: relationship to nine sexually transmitted organisms. Reprinted with permission from the American College of Obstetricains and Gynecologists (Obstet Gynecol 1982;142:962-7.) *Student test.

Table II. Studies of prevalence of recovered organisms according to birth control method used

	Study	oc	IUD	Other/barrier	None	Non-OC	All	p Value
Candidiasis	-							
Jensen (1970)	Prev	15%				5%		< 0.05
Catteral (1971)	Prev	99/200						
Bramley (1979)	Prev	68%				32%		< 0.001
Davidson (1985)	Prev	27.5%	25.5%	34.8%	27.7%		28.3%	
C. trachomatis				•				
Washington (1985)	Epid comp	3X				I		
Handsfield (1986)	Prev	11.5%	10.9%	3.5%	9.9%		9.3%	
Avonts (1990) about 2 yr	Ćohort	15%	4%			•		≤0.01
Bacterial vaginosis								
Avonts (1990) about 2 yr	Cohort	15%	45%					≤0.001
Trichomoniasis								
Bramley (1979)	Prev	37%				63%		< 0.002
Fouts (1980)	Prev	57/131			74/131			

candidiasis used birth control pills. A prevalence study by Bramley and Kinghorn⁴ in 1979 reported that 68% of OC users versus only 32% of users of other methods had yeast infection, a statistically significant increase. These three studies suggest an association between yeast infections and OC use. However, not all data support this association.

For example, a prevalence study by Davidson and Oates⁵ in 1985 examined the occurrence of yeast in the vaginas of unselected women. When subjects are chosen in this manner, one is impressed by the similarity in the percentage of yeast infections, regardless of the use of OCs, IUDs, other methods, or no method. Therefore the conclusion differs depending on the sample and how it is obtained.

It has been postulated that the mechanism by which yeast cells proliferate is linked with the immunologic competency of the host. Kalo-Klein and Witkin6 reported on the relationship of the menstrual cycle to lymphocyte proliferation and serum-induced germination of Candida. At midcycle, with estrogen predominance and a virtual absence of progesterone, they found the greatest proliferation of lymphocytes and the greatest percentage of ungerminated Candida. The next part of their study, which examined the effect of birth control pills, found that the percentage of ungerminated Candida tended to be higher overall in those patients taking birth control pills. This study suggests a mechanism by which Candida and birth control pills may be linked.

One of the earliest reports of C. trachomatis (Table II) by Washington et al.7 in 1985 reviewed 14 epidemiologic studies and found that in 12 studies the prevalence of C. trachomatis in the endocervix of OC users was three times that of nonusers of OCs. In a prevalence study by Handsfield et al.8 in 1986, the rates of Chlamydia were similar for all methods of contraception except for other or barrier methods, which had the lowest rate (3.5%). When they applied a stepwise multiple logistic model regression curve to use of no contraception or a nonbarrier method (i.e., the OC and IUD groups), they found a significantly increased odds ratio for those

Table III. Aerobes and anaerobes isolates

Bacteria	Barrier (n = 10)	OCs $(n = 10)$	$IUD \\ (n = 10)$
Aerobes	l [*	27	29*
Anaerobes	5*	20*	37*
Total No. of isolates	16	47	66

From Haukkamaa M, Standem P, Jousimies-Somer H, Siltonen A. Am J Obstet Gynecol 1986;154:520-4.

persons. Therefore this study reports an increased prevalence of *C. trachomatis* not only in the OC group but also in the IUD group. In a prospective study conducted over a 2-year period, Avonts et al.⁹ showed that the percentage cumulative chance of infection with *C. trachomatis* was significantly greater in the OC group (15%) versus the IUD group (4%) at the end of this time period.

This same cohort study by Avonts et al. reported on the occurrence of bacterial vaginosis (Table II). At the end of 2 years, 15% of OC users and 45% of IUD users had bacterial vaginosis, a significant difference. They concluded that symptomatic bacterial vaginosis was associated with IUD use, but asymptomatic bacterial vaginosis was associated with promiscuity. At 6 months, more persons in the IUD group than in the OC group had "pelvic inflammatory disease—like symptoms," but by 24 months there was no statistically significant difference between these two groups.

In 1979 Bramley and Kinghorn also reported on the decreased prevalence of *T. vaginalis* in OC users (37%) compared with users of other methods of contraception (63%) (Table II). In 1980 Fouts and Krauss dexamined clinical epidemiologic variables in women with and without vaginitis caused by *T. vaginalis*. Forty-four percent of the OC group had trichomoniasis, whereas 56% did not. Thirty-four percent of patients using no method of contraception also had trichomoniasis.

Obviously there are other organisms in the vagina, and Haukkamaa et al. "(Table III) compared the recovery of isolates from the endocervix of barrier method, OC, and IUD users. Seven of the 30 persons had only a single isolate recovered, whereas most had multiple isolates. In comparing the different methods, IUD users had significantly more aerobic colonies than barrier method users; also, both OC and IUD users had significantly greater numbers of anaerobic isolates than did users of barrier methods. Fundamentally, these data suggest that both the OC and the IUD users harbor more aerobic and nonaerobic organisms than users of barrier methods at the level of endocervix.

Table IV. Percentages of cultures positive for different bacteria in IUD users

	Group I, with symptoms	Group II, without symptoms	Group III, nonusers without symptoms
Samples from posterior f	ornix		
No. of patients Lactobacilli Anaerobic cocci Anaerobic rods G. vaginalis Streptococcus agalactiae	15 53.3 33.3 40.0* 26.7 26.7	19 73.7 31.6 10.5 10.5 5.3	19 63.2 10.5 21.1 10.5 10.5
Samples from the endom	etrium		
No. of patients Lactobacilli Anaerobic cocci Anaerobic rods G. vaginalis Steptococcus agalactiae	15 6.7 40.0 40.0 53.5 0.0	19 21.1 5.3† 10.5‡ 0.0§ 0.0	19 26.3 0.0‡ 10.5‡ 0.0 10.5
Samples from the IUD			•
No. of patients Lactobacilli Anaerobic cocci Anaerobic rods G. vaginalis Streptococcus agalactiae	15 20.0 13.3 26.7 26.7 6.7	19 42.1 5.3 5.3 5.3 5.3	N/A

From the Kivijarvi A, Jarvinen H, Gronroos M. Microbiology of vaginitis associated with the intrauterine contraceptive device. Blackwell Scientific Publications Limited. Br J Obstet Gynaecol 1984;91:917-23.

Kivijarvi et al.¹² studied three groups of persons (Table IV). Group I was IUD users, most of them Copper-7 or T users, with symptoms of vaginitis; group II was IUD users without symptoms. Both groups had these devices removed. Group III were non-IUD users without symptoms who were going to have them inserted at the time of this visit. Anaerobic rods were more prevalent in those IUD users with symptoms compared with those without symptoms when cultures were taken from the posterior fornix. However, a large number of organisms was found in all of these groups.

After cultures were taken, the cervix was then washed with saline solution, the speculum was removed and another put in, and the IUD was removed in a sterile fashion. An "endocyte," an instrument from France, was used to obtain a culture from the endometrium. There were more *G. vaginalis* in those women with

^{*}b < 0.01.

^{*}p < 0.05 versus group II.

 $[\]dagger p < 0.025$ versus group I.

 $[\]ddagger p < 0.01$ versus group I.

^{\$}p < 0.001 versus group I.

Table V. Bacteriologic comparison of prestudy with 6-month vaginal cultures

	Increase	Decrease	No change	+	-,	0
CVR (n = 20)	•			·····		•
Aerobes	10	2	8 .	0	0	0
Anaerobes	8	6	6	0	.0	0
Lactobacilli	3	0	. 3	3	2	9
Candida	0	0.	0	2 ,	1	17
N. gonorrhoeae	0	0	. 0	0	0	20
G. vaginalis	0	0	1	2	5	11
OC (n = 10)	1					
Aerobes	3	2	5	o ·	0	. 0
Anaerobes	3	1	6	0	0	0
Lactobacilli	1	0 .	2 .	4	0	3
Candida	0	0	0	3 ·	0	7
N. gonorrhoeae	0	0	0	0	0	10
G. vaginalis	0	0	0	0	2	8

From Roy S, Wilkins J, Mishell DR Jr. The effect of a contraceptive vaginal ring and oral contraceptives on the vaginal flora. Contraception 198124:481-491. Reprinted with permission of the copyright holder, Butterworth-Heinemann.

In magnitude (> or $<10^{1}$) of colony count from prestudy values.

CVR, Contraceptive vaginal ring; OC, oral contraceptive; +, appearance organism at 6 months when absent at prestudy; -, absence of organism at 6 months when present at prestudy; 0, absence of organism at prestudy at 6 months.

Table VI. Annual incidence per 1000 woman-years of first reports of cervical and vaginal conditions

*	,			U		
	Yr I		Yr 2		. Y-3	
Condition	Nor	TCu	Nor	TCu	Nor	TCu
Generalized questioning						
Cervical problems	3	. 6	0	0	. 4	0
Vaginitis/leukorrhea	98	124	124	226	143	169
Vulvitis	. 0	0	5	0	0	7
Other vaginal and vulvar symptoms	54	92	92	92	67	85
Total	155	222	221	318	214	261
Determined by physical examination					·	
Cervicitis	18	46	34	77	4	28
Cervical erosion, lesion	26	64	34	41	13	7
Other cervical	19	14	24	21	22	42
Total cervical	63	124	92 '	139	39	77
Vaginitis	. 84	139	114	. 180	63	92
Leukorrhea	51	133	148	370	117	240
Vulvitis	6	0	10	0 .	0	14
Other vaginal or vulvar	10	20	7	21	9	7
Total vaginal vulvar	151	292	279	571	189	353

From Sivin I. Clinical effects of Norplant subdermal implants for contraception. In: Mishell DR Jr, ed. Long-acting steroid contraception. New York: Raven Press, 1983;2:89-116.

NOR, Norplant; TCu, Copper T.

symptoms compared with those without symptoms. Anaerobic rods and cocci were more prevalent in those patients with symptoms than without symptoms. Multiple organisms were recovered from the endometrium from several individuals not using an IUD and without symptoms. There were percentages of greater recovery of all organisms except lactobacilli from the IUDs of those with symptoms than without, although these differences did not reach statistical significance.

A recent study by Roy et al. 13 compared the recovery of organisms from users of the contraceptive vaginal ring and women taking OCs. The contraceptive vaginal ring is a silicone rubber device in the shape of a donut, with the active ingredient in the middle portion of a three-layer shell design. This vaginal ring releases levonorgestrel and estradiol. The comparison group consisted of individuals taking OCs containing levonorgestrel and ethinyl estradiol. Cultures were obtained from the posterior fornix before randomization to this therapy and then at the end of 6 months of therapy. In Table V¹³ the numbers of subjects are presented in whom there was a 10¹ order increase or reduction in cultures, no change, those who did not have a positive culture at the beginning but had it at the end, those who had it at the beginning, lost it at the end, and then those in whom no change occurred, or positive culture was never present. No demonstrable change in the pattern of recovery for these types of organisms occurred in those patients who used the vaginal ring or OCs.

Norplant, a subdermal method of birth control, releases levonorgestrel from six Silastic capsules, each 3 cm in length. They are inserted subcutaneously in the medial aspect of the upper arm and release a small amount of levonorgestrel into the circulation. This method is being used worldwide presently and has recently been approved in the United States by the Food and Drug Administration. Some data are available with respect to the issue at hand; namely, cervical problems, vaginitis, leukorrhea, vulvitis, and other vaginal or vulvar symptoms. In 1983 Sivin¹⁴ reported on 1, 2, and 3 years of use of Norplant, a 5-year method, and compared it with the Copper-T IUD, which at that time was a 3-year method (Table VI). Norplant users had approximately one third fewer complaints compared with Copper-T users. When these individuals were actually examined, Norplant users had about 40% to 50% of the vaginal or vulvar complaints compared with IUD control subjects.

Comment

The studies that would provide answers for the purpose of this review are not available. However, those studies that are available suggest that OC use probably does not change the risk of candidiasis, may increase the risk of C. trachomatis and in fact appears to reduce the risk of bacterial vaginosis and trichomoniasis compared with other nonbarrier methods of birth control. IUD use does not change the risk of candidiasis or C. trachomatis; indeed, the incidence of the latter may be lower than in the OC users. However, the rate of bacterial vaginosis may be increased compared with OC users. Interestingly, the link between IUD use and "pelvic inflammatory disease-like symptoms" suggested by Avonts et al.9 appears to be related to promiscuity. This confirms the observations with IUD use: namely, that there may be a risk of infection of the upper genital tract immediately after IUD insertion; thereafter, the risk is the same as acquiring a sexually transmitted disease.15 The report by Kivijarvi et al.12

supports the concept of Hemsell et al.¹⁶ that even women without symptoms may harbor microorganisms in the upper genital tract without evidence of disease. The contraceptive vaginal ring and Norplant appear not to be related to any particular vaginitidies. In conclusion, given the constraints of the published studies, there does not appear to be any direct association between any of these methods of contraception and the occurrence of the various vaginitidies.

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Cytolytic vaginosis

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Cytolytic vaginosis, a not uncommon condition, is frequently misdiagnosed because it is confused with *Candida*. Many practitioners rely on their clinical judgment alone rather than the use of high-quality microscopes and the results of a wet smear. Compounding the problem of misdiagnosis is that patients assume that their symptoms are caused by a yeast infection, which results in telephone requests for medication from their physicians instead of an office consultation. Cytolytic vaginosis is characterized by pruritus, dyspareunia, vulvar dysuria, and cyclical increase in symptoms more pronounced during the luteal phase. Diagnostic criteria include a high risk of suspicion; the absence of *Trichomonas, Gardnerella*, or *Candida* on wet smear; an increased number of lactobacilli; a paucity of white blood cells; evidence of cytolysis; and the presence of discharge; and a pH between 3.5 and 4.5. Treatment entails use of sodium bicarbonate douches. (AM J OBSTET GYNECOL 1991;165:1245-9.)

Key words: Vaginosis, vaginitis, Candida, Lactobacillus

Cytolytic vaginosis is a newly coined name for an old ailment, Döderlein's cytolysis, which is quite common. In our experience, *Lactobacillus* overgrowth and candidiasis are frequently confused, and many patients referred for treatment of "chronic yeast infections" are singularly free of *Candida*.

The fault lies with the clinical practice of relying on color and physical characteristics of a vaginal discharge to make a diagnosis. The errors are further perpetuated by the so-called "easy diagnosis of vaginitis" charts that use color and consistency of discharge, as well as symptomatology as diagnostic criteria. Too many of us fervently believe that we can accurately diagnose a case of "Monilia" and that "after 20 years in practice, I do not need a microscope to diagnose an ailment that I have seen a thousand times." In our practice, if we are unable to make a diagnosis, we instruct the patient to refrain from sexual relations and not to use vaginal medications or douches. She is further instructed to return to the office in 48 to 72 hours. Using this protocol, we are almost always able to make an accurate diagnosis.

We challenged the accuracy of the "eyeball" diagnosis at the 1985 national meeting of the American College of Obstetricians and Gynecologists.' In a scientific exhibit, after viewing a series of slides, participants were asked to register a diagnosis based solely on color, consistency, and physical characteristics. The average score of those physicians was 30%.

Often a patient manipulates her physician into pro-

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viding medication for a "yeast infection" that was never clinically examined, but, rather, diagnosed on the telephone. "The telephone," as stated by Friedrich, "is neither a diagnostic nor a therapeutic tool and the temptation to use it as such should be resisted."2 Too many patients and far too many physicians believe that if you have ever had or been told that you had a yeast infection, then any further vaginal discharge, accompanied by pruritus, automatically should be treated as Candida. The recent Food and Drug Administration decision to allow the over-the-counter purchase of clotrimazole and miconazole will undoubtedly add to the tremendous therapeutic confusion and encourage the overdiagnosis and incorrect treatment of so-called Candida. The concept of self-treatment will also lead to failure to diagnose and treat the more dangerous sexually transmitted diseases. Blind diagnosis compounded by conjectural pharmacology is akin to playing vaginal roulette.3

It is imperative to point out that even if a discharge is white, cheesy, and found to test in the pH range of 3.5 to 5.0, it still may not be *Candida*. Many women, discouraged by the failure of traditional medical treatment, follow a cult-like, self-help group which employs garlic-impregnated tampons, myrrh pastes, and acupuncture. They also use herbal drugs, yogurt, and massage in an attempt to alleviate debilitating symptoms. The standard dictum of "when in doubt, douche with vinegar" has become widely regarded as a cure-all. All of these exercises are futilely carried out to restore the *Lactobacillus* as the defender of vaginal health.

For nearly a century, physicians and microbiologists have championed the Döderlein's bacillus as the savior of women. Cruickshank and Sharman⁴ described three grades of vaginal health that were dependent on

the number of Döderlein's bacilli in the vagina. Fifty years ago, Weinstein reported that there seemed to be no correlation between vaginal pathology and the presence or absence of the Döderlein organism, with the exception of the gonococcus and the trichomonad, in which case, Döderlein's organism was never demonstrated. The earlier philosophy of correlating vaginal health with the numbers of lactobacilli is no longer a viable hypothesis. Sobel, as well as Larsen and Galask, concur with Weinstein's original findings.

While the authors recognize that chronic candidiasis is a definite entity, the majority of patients referred to our practice are found to be suffering from the entity previously described as Döderlein's cytolysis. From its inception, cytology has recognized the phenomenon of cytolysis. Bibbo⁸ reported cytolysis as a common finding on Papanicolaou smears and suggested distilled water douches to raise the pH. She did not, however, describe the condition as a clinical entity. Inasmuch as only a few species of lactobacilli are, in fact, Döderlein's bacilli, it became apparent to the authors that the term Döderlein's cytolysis is a misnomer and that a far more appropriate description is cytolytic vaginosis.⁹

The woman with a chronic, uncured vaginitis is frequently advised to seek psychiatric counseling, or even worse, to find the "ultimate cure" by having her cervix and uterus removed. Although Döderlein's bacillus was first described in 1892,10 we now know that the organism bearing Döderlein's name is not a uniform entity but is equally applied to the Lactobacillus and other rods.11 The Lactobacillus is a pleomorphic, grampositive, aerobic or a facultative anaerobic, non-sporeforming organism. As many as 80 different species have been described. Numerous investigators have observed that the Lactobacillus is found in normal vaginal secretions and is associated with a high degree of acidity. Rakoff and associates questioned whether the lactic acid in the vagina resulted from conversion of carbohydrates by certain bacteria, that is, the Döderlein bacteria.12 They further asked whether there was enzymatic fermentation of carbohydrates to lactic acid by other mechanisms irrespective of the Lactobacillus. 12

Many early studies have confirmed that the vagina is rich in glycogen; in fact, it is second only to the liver in total glycogen concentration. The question of whether the *Lactobacillus* is capable of fermenting glycogen to lactic acid is of continuing debate. Cruickshank and Sharman, as well as Wylie and Henderson, shave demonstrated that some strains of *Lactobacillus* are capable of directly fermenting glycogen, and that the "vaginal acidity is brought about, in part, at least, by the vaginal *Lactobacillus*." They further demonstrated that the majority of lactobacilli strains are unable to

ferment glycogen directly but are capable of fermenting glucose. ¹³ Stewart-Tull¹⁴ hypothesized that the "vaginal *Lactobacillus* lacked either phosphorylase or glucosidase" and believed that high acidity in the vagina was caused by the breakdown of glycogen to free glucose by muscle phosphorylase and glucosidase. The free glucose can then, in turn, be fermented by the *Lactobacillus*.

Many species of lactobacilli ferment glucose predominantly to lactic acid with only a trace amount of other products by homolactic fermentation, whereas others are heterolactic fermenters that produce a mixture in which half of the glucose is converted to lactic acid, with the remainder forming a carbon dioxide, alcohol, formic acid, or acetic acid.¹⁵

The normal flora of the vagina exists in an everchanging arena of physiologic checks and balances. The inhibition of the growth of one species of bacteria by liberation of H₂O₂ by another species is a well-known mechanism of bacterial control.16 Lactobacilli and other lactic acid producers lack heme and are incapable of using the cytochrome system to form H2O. Lactobacilli use flavoproteins which convert O2 to H2O. Flavoprotein enzymatic action, combined with the lack of the heme protein, catalase, produces large amounts of H₂O₂. This excess formation may kill or inhibit other bacteria, especially those that lack or have suboptimal H₂O₂ scavenging enzymes, that is, catalase peroxidase. The bactericidal property of H2O2 is markedly enhanced by the enzyme peroxidase in the presence of a halide ion. The peroxidase enzymes that function in this manner are found in milk and saliva, neutrophils, monocytes, and eosinophils, and in genital tract secretions, including cervical mucus.17

The concept of cytolytic vaginosis has not been adequately explored and continues to raise questions. If this is a pathologic entity, it is natural to question why more women are not afflicted with this overgrowth of lactobacilli. The answer may very well lie in the symbiotic relationship among various naturally occurring bacterial flora and the differences in their fermentation by-products caused by the differentiation of species of *Lactobacillus*. To date, intensive bacteriologic studies, including quantitative studies of vaginal flora, have not been helpful in clarifying the issue. More investigation is warranted if an answer is to be found.

There is much confusion in the literature regarding the role of vaginal cultures. Kaufman and Friedrich have demonstrated that the wet smear accurately identifies the most common organisms: Candida, Trichomonas, and Gardnerella. They also noted that the Lactobacillus was easily identifiable through a wet mount.¹⁸

Except to confirm a diagnosis of chlamydia, gonor-

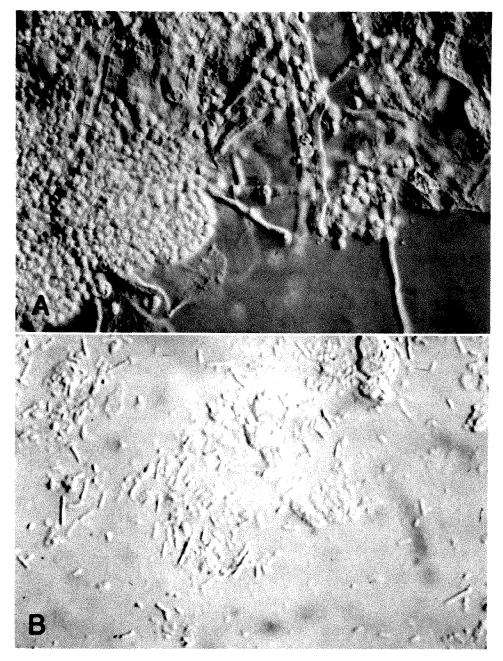


Fig. 1. Symptomatically confused with candidiasis, cytolytic vaginosis looks much different with the Nomarski microscope. **A,** *Candida albicans* seen at 200 power. Note buds and pseudohyphae. **B,** Cytolytic vaginosis seen at 400 power. Note increase in lactobacilli.

rhea, or mycoplasm, we rarely use cultures for the diagnosis of vaginitis. To date, we have personally examined more than 10,000 wet smears with Phase and Nomarski microscopy and are firmly convinced that the microcosm of the vaginal flora is better studied in its natural milieu than in artificial laboratory cultures.

We suggest criteria for the evaluation of a wet smear:

- 1. A 5-minute search under 200 to 400 power magnification is required before stating a slide is neg-
- ative. The microscope should be of modern quality and a cover slip of proper thickness is an essential part of the system. Examination under oil immersion is almost never needed.
- 2. The wet smear should be taken from the vaginal side walls or the pool or both. It should not be taken from the cervix, except for cultures, and once obtained, should be examined promptly by an experienced examiner. The cotton or Dacron

swab should be mixed with four or five drops of normal saline or Ringer's lactated solution and placed in either a test tube or a paper cup. The sample should then be applied directly to the slide. An undiluted smear is often far too thick to interpret properly and dries too easily.

- 3. We find that potassium hydroxide is seldom necessary to diagnose *Candida* in the wet smear if the observer has spent time in practice reading. Potassium hydroxide frequently damages the objective lenses of the microscope by etching the glass and causing a loss of resolution and contrast in the specimen.
- 4. The Phase or Nomarski microscope gives an entirely new perspective in evaluating the wet smear (Fig. 1). The study of living vaginal organisms is, in our opinion, the most logical way of studying the ecology of the female lower genital tract. There is no comparison with the fixed slide, as in Papanicolaou preparations or Gram's stains, with that obtained in vivo by Nomarski or Phase equipment. We strongly urge gynecologists to become acquainted with this modern method of microscopy.

Cytolytic vaginosis is an easily diagnosed, easily treated entity that requires no elaborate laboratory tests, uses low-cost treatment with agents found in most households, and is totally without risk to the patient. Our criteria for diagnosis of this condition include a high index of suspicion; absence of Trichomonas, Gardnerella, or Candida on wet smear; an increased number of lactobacilli (often adherent to the intermediate epithelial cell, and to the inexperienced observer are often confused with the clue cell of bacterial vaginosis); a paucity of white blood cells; evidence of cytolysis with bare or naked intermediate nuclei; discharge (which may be white, frothy, or cheesy); and a pH between 3.5 to 4.5. The patient who presents with cytolytic vaginosis complains of pruritus, dyspareunia, vulvar dysuria, and cyclic increase in symptoms that are more pronounced in the luteal phase. She generally carries a shopping bag full of partially used medications, many of which are generic clones, that have failed to cure or even alleviate her condition.

Treatment for cytolytic vaginosis consists of increasing the pH of the vagina by means of sodium bicarbonate douches. We recommend a douching solution of 30 to 60 gm of sodium bicarbonate to 1 L of warm water two to three times per week and then once or twice a week as needed. The patient can also be instructed to start douching 24 to 48 hours before the anticipated onset of symptoms.

The exogenous addition and increased concentration of lactobacilli to reestablish the so-called ecologic bal-

ance of the vagina has no scientific relevance. This is also true for the indiscriminate use of vinegar-andwater douches. To add more lactobacilli or to use vinegar douches that further lower the vaginal pH in a patient who already exhibits a low vaginal pH, as well as an overgrowth of *Lactobacillus*, will only increase the symptoms. Yogurt, instilled into the vagina, is also of dubious value as therapy for vaginitis.¹⁹

It appears that the *Lactobacillus*, either alone or in conjunction with other bacteria, seems to be responsible for the signs and symptoms of cytolytic vaginosis. Regardless of the mechanism, a clinical entity exists that causes discharge and burning and is characterized by a profusion of lactobacilli with dissolution of the cytoplasm of the intermediate vaginal epithelium. The authors believe that use of the more sophisticated Phase and Nomarski microscopes will result in greater cost containment and more prompt and accurate diagnosis. This will reduce the reservoir of patients who have become emotionally crippled by this nonlethal but debilitating condition called cytolytic vaginosis.

There are many unanswered questions concerning this entity. We realize that there are insufficient data for statistical evaluation. The scope of the study has been directed toward qualitative interpretation rather than quantitative hard data. The question of pH change and its duration, as provided by baking soda douches, needs to be addressed. The authors plan such an investigation.

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Postpartum vaginal atrophy

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Postpartum vaginal atrophy has been briefly noted without adequate clinical description. We present a study of 215 puerperas at a scheduled 4-week postpartum visit, investigating colorpHast vaginal pH, vestibular cotton swab testing, vaginal wall tenderness, and both fixed and wet mount cytologic studies. Thirty-seven patients with vaginal atrophy (17.2%) had a mean pH of 6.55 with a mean maturation index of 49.2% parabasal, 46.5% intermediate, and 4.3% superficial cells. Eighty patients (37.2%) served as control subjects, with a mean maturation index of 0.3% parabasal, 73.6% intermediate, and 26.1% superficial cells, and a mean vaginal pH of 4.68. Ninety-eight patients (45.6%) were excluded for multiple confounding conditions, the most common of which was bacterial vaginosis in 37 (17.2%). This study demonstrates the existence of postpartum vaginal atrophy, notes lactation as an apparent causal factor, and establishes a statistically significant (p < 0.0001) relationship between vaginal pH and maturation index. Further studies are needed to confirm therapeutic efficacy of estrogens. (AM J OBSTET GYNECOL 1991;165:1249-54.)

Key words: Atrophic vaginitis, vaginal maturation index and pH, lactation, vestibulitis

From rupture of membranes to resumption of ovarian function, the vulvovaginal ecosystem is subjected to an alkaline environment, relative estrogen deprivation, and the mechanical trauma of childbirth. Although various elements of these changes have occasionally been noted in the literature, ¹⁻⁶ very little clinical information about the puerperal atrophic changes in the vulva and vagina is available. Our earlier pilot study⁷ evaluated

112 patients at 6- to 14-week postpartum visits. Fourteen patients (12.5%) had dyspareunia, vaginal stinging, dysuria, vaginal tightness, tenderness on speculum examination, vaginal color change, or scant discharge. Their mean vaginal pH was 6.3 compared with a control group at 4.7, establishing elevated pH as a sign of this condition. Three patients noted similar symptoms after previous pregnancies. Eleven of the 14 patients were treated with intravaginal estrogen, and the condition resolved within 28 days. The conditions of three untreated patients resolved in 56 to 180 days with oral contraception or resumption of ovarian function despite continuing lactation.

Progressing from the shortcomings of that study, this effort was initiated at a scheduled 4-week postpartum

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Table I. Vaginal pH on initial visit—colorpHast

pН	Atrophic	%	Controls	%	Total
4.0	0	0.00	12	15.00	12
4.4	Õ	0.00	29	36.25	29
4.7	Ŏ	0.00	7	8.75	7
5.0	ŏ	0.00	17	21.25	17
5.3	3	8.11	9	11.25	12
5.5	ő	0.00	5	6.25	5
5.7	3	18.11	0	0.00	3
5.9	4	10.81	ì	1.25	5
6.2	3	8.11	0	0.00	3
6.5	9	5.40	Ö	0.00	2
	$\frac{2}{22}$	59.46	ő	0.00	22
7.0 Total	37	33.10	80	****	117

No statistical inference drawn.

visit that investigated vestibular cotton swab testing,⁸ colorpHast vaginal pH,⁹ vaginal wall tenderness, and both fixed and wet mount cytologic studies. Elevated vaginal pH was used as the most objective of the previously recognized inclusion criteria. The goals were to more clearly appreciate the clinical parameters of vulvovaginal postpartum atrophy, to establish the significance of the relationship between the cytologic maturation index (MI) and vaginal pH, and to suggest treatment.

Material and methods

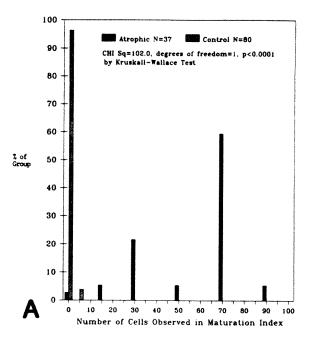
Two hundred fifteen puerperas delivered in a solo private Philadelphia obstetric practice who returned to the office for a scheduled 4-week postpartum visit between March 1989 and June 1990 were entered into this study. Noting a published statement 10 on absent reported risk, the protocol and ongoing study were approved and monitored by the institutional review board of the Pennsylvania Hospital, Philadelphia, Pa. A thorough interval history was taken with special attention directed at eliciting vaginal stinging or burning, vaginal tightness, dysuria, and dyspareunia, both insertion and deep thrust, and also postcoital dyspareunia. Physical examination included breasts, abdomen, cervical Papanicolaou smear, and bimanual examination. We also incorporated vestibular cotton swab testing, colorpHast anterolateral vaginal wall pH, elicitation of tenderness on gentle scraping of the lateral vaginal walls with a plastic scraper, lateral vaginal wallfixed cytologic specimens later stained with the Papanicolaou technique, fresh specimens of the lateral vaginal wall examined immediately in normal saline wet mount preparation, and urine culture and sensitivity.

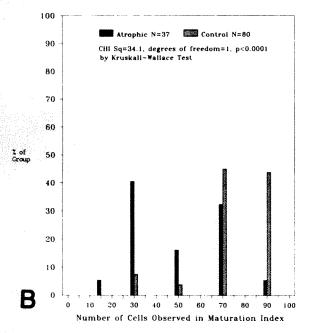
Considering that all patients recently had negative test results or had been successfully treated for syphilis, gonorrhea, and chlamydia during pregnancy, no additional effort to detect these conditions was made. Inclusion criteria for this study were a vaginal pH greater than or equal to 5.3 and at least one symptom (dysuria, tightness, stinging, burning, or any dyspareunia) and/or at least one sign (scant vaginal discharge, red or pallid vaginal mucosa, at least a two-site positive vestibular cotton swab test, or tenderness elicited on gentle scraping of the lateral vaginal wall). Because of the complexity of the vulvovaginal milieu, great care was taken to attempt to exclude all other confounding variables. Exclusion criteria for this study were bacterial vaginosis, trichomoniasis, candidiasis, cytolytic vaginosis, cervical intraepithelial neoplasia, human papillomavirus disease, active herpes simplex virus, symptomatic cystitis, incomplete healing at episiotomy or laceration sites, ovulation (in nonlactating patients who came to the office more than 40 days post partum), and other mechanical factors, such as active bleeding, douching, or coitus within 6 hours. Air-dried smears and endometrial, cervical, or introital-contaminated smears were also excluded. Control subjects were all remaining patients neither included nor excluded. The cytologic part of the study was performed independently and blindly in Gainesville, Fla.

All included patients were provided conjugated estrogen vaginal cream at a dose of 2 gm per vagina twice a week and instructed to return at 14-day intervals until no evidence of atrophy remained. Excluded patients were treated appropriately where necessary. Statistical analysis used the modifications of the χ^2 test as noted below.

Results

A total of 215 patients returned for their scheduled postpartum visit. Thirty-seven patients (17.2%) merited placement in the included, atrophic group. This group had a mean vaginal pH of 6.55 with a mean MI of 48.19% parabasal, 46.49% intermediate, and 4.32% superficial cells. The symptoms noted in this group in-





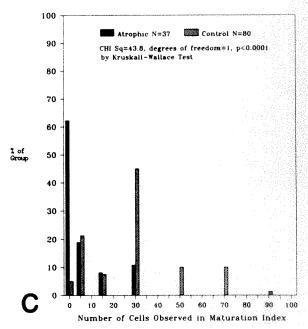


Fig. 3. Graphic representations of cytologic data in the study group by MI component.

cluded dyspareunia in 8 of 10 patients (80%) who attempted coitus, 6 (16.2%) had vaginal stinging, 4 (10.8%) had vaginal tightness, and 1 (3%) had dysuria. Four (10.8%) had experienced similar symptoms after previous pregnancies. Epithelial vestibular vaginal color change was the most frequent sign elicited from the included group of 35 of 37 patients (94.6%). Thirty-three patients showed red change (Fig. 1, p. 1227), whereas 2 had pallid change (Fig. 2, p. 1227). Ten-

derness of the lateral vaginal wall was elicited in 33 patients (89.2%) of this group. Vestibular tenderness by swab testing was present in eight subjects (21.7%) of this group.

Eighty patients (37.2% of total) remained in the control group. This group had a mean vaginal pH of 4.68 with a mean MI of 0.31% parabasal, 73.63% intermediate, and 26.06% superficial cells. Eighteen (22.5%) of these control subjects had an elevated pH between 5.3

Table II. Infant feeding

	Nursing	Formula	Total
Atrophic			
No.	25	12	37
%	67.57	32.43	
Controls			
No.	10	70	80
%	12.5	87.5	
TOTAL	35	82	117

p < 0.0001 by Pearson.

and 5.9 without signs or symptoms, whereas 29 subjects (36.3%) had signs or symptoms without an elevated pH. The symptoms noted in this group included dyspareunia in 4 of 31 patients (12.9%) who attempted coitus and vaginal tightness in 4 (5%). Signs elicited in this group included two subjects (2.5%) with red vaginal mucosa, three (3.8%) with lateral vaginal wall tenderness, and eight (10%) who had vestibular swab tenderness.

Ninety-eight patients (45.6% of total) were excluded for the following reasons: 37 (37.8%) for bacterial vaginosis, 21 (21.4%) for unreliable cytologic findings or pH, 7 (7.1%) for symptomatic cystitis, 7 (7.1%) for cervical intraepithelial papillomavirus disease, 7 (7.1%) for poor healing at episiotomy or laceration sites, 6 (6.2%), who returned between 40 and 70 days post partum for signs of imminent ovulation, 6 (6.2%) for trichomoniasis, 4 (4.1%), who came at a mean 53.5 days post partum for candidiasis, and 3 (3.3%) for cytolytic vaginosis (Döderlein cytolysis). Fifteen patients had multifactorial exclusion criteria.

A comparison of the vaginal pH used as the selection criteria between the inclusion and control groups is given in Table I. Comparison of the MI profiles between the two groups in Fig. 3, A through C, demonstrates a marked left shift, with increasing percentages of parabasal cells and decreasing superifical cells, which defined the inclusion group as atrophic. The infant feeding comparison described in Table II demonstrates a significant correlation between lactation and postpartum vaginal atrophy. Of note, only 67.6% of nursing mothers had vaginal atrophy, whereas 32.4% of patients with atrophy were not lactating. Comparison of route of delivery in Table III demonstrates no significant difference in development of postpartum vaginal atrophy. The therapeutic response to intravaginal conjugated estrogen cream is noted in Table IV. Although all treated patients had resolution within 40 days, spontaneous remission and decreasing lactation may account for a portion of the improvement. The vaginal

Table III. Route of delivery

	C/S	Vaginal	Total	
Atrophic				
No.	6	31	37	
%	16.22	83.78		
Control				
No.	18	62	80	
%	22.5	77.5		
TOTAL	24	93	117	
%	20.51	79.49		

C/S. Cesarean section.

estrogen cream did not cause any reported systemic effects in either nursing mothers or their infants.

Comment

Kauffman et al.11 define vaginal senile atrophy as a condition with a left shift in the cytologic vaginal MI and increasing percentages of intermediate and parabasal cells and decreasing superficial cells caused by withdrawal of estrogen. They note most patients deny symptoms, although dysuria, external burning, tenderness, dyspareunia, slight discharge, and pruritus may accompany this condition. Signs include vaginal mucosal color change from pink to diffuse redness to pallid, traumatic vulnerability to coitus or speculum examination, petechiae, and the eventual disappearance of folds and rugae. A scant discharge is present with increased parabasal cells, white blood cells, and absent lactobacilli. Lateral vaginal pH is between 6 and 7. A subset of vaginal atrophism resulting from radiation therapy causes more severe changes. The causes include direct radiation and pressure damage and irradiation castration. Topical and/or systemic estrogen is the treatment of choice for both conditions.

In 1944 Rakoff et al.1 noted that the decrease in ovarian activity during perimenopause was accompanied by a rise in vaginal pH progressing to neutral (7) levels during menopause. In 1955 Lang² observed that the puerperal vagina is bathed with alkaline lochia. In 1958 Kauffmann et al.3 noted parabasal cells in postpartum cervical cytologic studies analogous to those seen in senile atrophic vaginitis. Masters and Johnson⁴ in 1966 published observations on visual examination of three lactating and three nonlactating mothers at 4, 8, and 12 weeks post partum. They noted a decrease in physiologic response, flattened rugal patterns, and a "senile"-appearing light pink color change at 4 and 8 weeks. They also noted that nursing patients had a "steroid starvation pattern." Recovery was appreciated at 12 weeks. They also conducted 12-week interviews

 $[\]chi^2$ test with 1 degree of freedom.

p = 20.59 (not significant) by χ^2 test with one degree of freedom.

Table IV. Topical estrogen treatment response

Mean days No. treated	Mean days	Mean days		Mean	MI		
	post partum	Nursing	pH	Parabasal	Intermediate	Superficial	
37 28 9	0 18.82 38.57	31.54 50.36 70.11	25 (67.57%) 15 (53.57%) 4 (44.44%)	6.55 5.08 4.82	49.19 5.89 0.00	46.49 70.72 71.12	4.32 23.39 28.88

One patient refused therapy.

No statistical inference drawn.

Placebo effect uncontrolled.

with 101 puerperas in whom 11 (10.9%) noted vaginal tightness and 47 (46.5%) noted irritation or dyspareunia. They concluded "... for at least 4 weeks after delivery, all women are essentially castrates so far as ovarian function is concerned...." More recently, Friedrich⁵ noted alkaline change in the vagina with dyspareunia during lactation and suggested twice-weekly conjugated estrogen vaginal cream. Giuntoli et al.6 observed vaginal colposcopic and cytologic atrophic changes during the postpartum period and noted their continuation for prolonged periods in nursing mothers. The postpartum condition described in the present study fulfills nearly all of the criteria of Kauffman et al.11 for vaginal atrophy and should be considered as the third subset of that entity. Although white blood cells are frequently present in the puerperal vagina, they may be of lochial origin, making the use of the term "vaginitis" questionable. Although the pH selection cutoff at 5.3 may have been somewhat low, scheduling the initial postpartum visit at 4 weeks produced clearer results than our pilot study.7 From the exclusion group, we note the 17.2% rate of bacterial vaginosis is comparable with similar studies^{12, 18} with 14% and 22% rates. Of clinical interest, fixed stained cytologic studies were almost twice as effective in the diagnosis of this condition, although the homogenous discharge and bacterial corkscrew wet mount patterns described by Thomason et al.14,15 were not used. The heavy alkaline cervical discharge that accompanies spinnbarkeit and ferning during the actual hours of ovulation invalidating these vaginal pH readings has received scant recent notice in the literature. The reliable colorpHast, indicator strips, and an improving appreciation of the vaginal ecosystem may facilitate these studies. The relative absence (1.86%) and delayed appearance (53.5 mean postpartum days) of Candida in these patients may reflect vaginal alkalinity as an inhibiting factor in this infection.

The comparison of control and atrophic patients by type of infant feeding (Table II) indicates a statistically significant (p < 0.0001) correlation between vaginal

atrophy and nursing. That this relationship was not universal may be from variations in individual estrogen receptors and peripheral conversion of androstenedione to estrone in fatty tissue. Comparison of symptoms seems to indicate that the dyspareunia present in 12.9% of control subjects and 80% of atrophic patients attempting coitus may have some diagnostic significance. However, several signs were even more promising. Mucosal red or pallid color change was present in 94.59% of the atrophic group versus 2.5% of control subjects. Lateral vaginal wall tenderness was appreciated in 89.19% of atrophic patients versus only 3.75% of control subjects. The color changes are caused by a thinning of the vaginal mucosa. The tenderness in the usually poorly sensitive lateral vaginal wall may also reflect a thinner vaginal mucosa exposed to an irritative alkaline environment. The vestibular swab tenderness seen in 21.62% of patients with atrophy but also 10% of control subjects seems less specific. First appreciated in the enigmatic vulvar vestibulitis, this sign may be the final common pathway for many different types of vestibular irritation. Its presence in the alkaline puerperal vestibule may indicate a vulvar component to vaginal atrophy. However, more data will be required to be convincing.

The similarities in both groups in mode of delivery (Table III) would argue that mechanical trauma is not a statistically significant factor in vaginal atrophy. The therapeutic response noted in Table IV is gratifying but uncontrolled for placebo effect. Double-blinded placebo/estrogen vaginal cream studies will be required to demonstrate therapeutic effectiveness.

Of all the results in this study, the relationship between vaginal pH and MI may be the most significant not only in the p value (p < 0.0001) but also in broader measure. The MI has been criticized16 for lack of precision, sensitivity, specificity, and accuracy. However, the studies17, 18 that generated this criticism were based on menopausal symptoms, which are denied by most patients and full of subjective variation. In vaginal atrophy, symptoms may be considerably less reliable than the signs (color change, pH, MI, scant discharge, absent lactobacilli, and vaginal tenderness). We have been able to demonstrate a significant relationship between vaginal pH and MI. Using similar methods, other vaginal relationships may be amenable to study. Indeed, the development of an inexpensive (colorpHast strips at 8 cents each) indicator for estrogen replacement that uses vaginal pH would seem worthwhile. Finally, this study taught us that accomplishment of reproducible scientific work dealing with the human vagina necessitates separating out many dependent variables in an attempt to isolate those factors that initiate change.

We gratefully acknowledge the contributions of our colleagues in the International Society for the Study of Vulvar Disease, on whose shoulders this work stands. In particular, Raymond H. Kauffmann, MD, and the late Eduard G. Friedrich, Jr., MD, were mentors for the experimental design. Greg Maislin, MS, MA, of Biomedical Statistical Computing, performed the statistical calculations. Michael J. Warhol, MD, and Thomas V. Sedlacek, MD, provided manuscript review. Charlene M. Lewis, IAC, ASCP, CT, provided cytotechnologist support.

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Vaginal cancer: The role of infectious and environmental factors

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Primary cancers of the vagina are rare. They comprise 1% to 2% of all gynecologic malignancies and occur predominantly in older women. The diagnosis of primary carcinoma of the vagina requires that the cervix and vulva be intact and that no clinical evidence of other primary tumors exist. Approximately 90% of all vaginal tumors are squamous cell in type on histologic examination. Adenocarcinoma, which is much less common (2% to 4%), is seen primarily in younger women with in utero exposure to diethylstilbestrol. In addition to exposure to diethylstilbestrol, other environmental factors have been associated with the development of vaginal tumors, including chronic irritation from pessaries, previous hysterectomy for benign disease, immunosuppression therapy, cervical irradiation, and endometriosis. Infectious causes seem to play an even more pernicious role in vaginal cancer. The two agents most often implicated are herpes simplex virus and human papillomavirus. These viruses appear to serve as cofactors in the inducement of various genital cancers, working together or with environmental agents such as diethylstilbestrol and host-related genetic abnormalities. The prognosis of vaginal cancer depends on the stage of the disease, with an overall 5-year survival rate of 80% to 90% for early stages. (Am J Obstet Gynecol 1991;165:1255-62.)

Key words: Vaginal cancer, herpes simplex virus, human papillomavirus, diethylstilbestrol, cervical cancer

Of all genital cancers in women, primary carcinoma of the vagina is second only to cancer of the fallopian tubes in its rarity. Vaginal tumors account for approximately 1% to 2% of all gynecologic malignancies, with an estimated incidence of 300 to 700 new cases each year. Secondary malignancies are, by contrast, quite common and make up 80% to 90% of all tumors in this site. This is probably because of the anatomic location of the vagina, which lies dorsal to the urinary bladder and ventral to the rectum. Primary tumors in these locations can involve the vagina by direct spread or by lymphatic dissemination.

Diagnosis and presentation

Primary vaginal cancer is a disease of older women, with the peak incidence occurring during the sixth and seventh decades. Only about 15% of patients are 50 years of age or younger.⁴

Symptoms of the disease may vary. The most common clinical complaint is that of irregular bleeding, but leukorrhea, or especially in advanced cases, pelvic pain, can also occur. However, many patients have no symp-

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toms, and a diagnosis is made during the investigation of other conditions, such as uterine prolapse, rectal or urinary fistulas, an abnormal Papanicolaou smear, or less commonly by a palpable mass. Tumors are usually located in the vault or upper third of the vagina with a slight predilection for the posterior wall. To qualify as a primary vaginal squamous cell carcinoma, some authors require that the cervix and vulva be without tumors and that no clinical evidence of tumors exists elsewhere.

Tumor traits

The upper vagina and cervix share an identical stratified squamous epithelium, and each structure is highly sensitive to hormonal stimulation. Therefore it is not surprising that on histologic examination, lesions in the upper vagina closely resemble those found in the cervix, regardless of whether the tumor is in situ or invasive.

The histologic appearance of vaginal tumors varies. Approximately 90% are squamous cell carcinomas and may present as either in situ or invasive lesions.²

Adenocarcinomas account for 2% to 4% of the tumors and are typically seen in patients who have been exposed to diethylstilbestrol (DES). Other primary malignant tumors include malignant melanoma, sarcomas, and embryonal rhabdomyosarcoma in children.

Vaginal squamous cell carcinoma may have different

Table I. Predisposing factors in the pathogenesis of vaginal cancer

Strong association
HPV (may be a cofactor with HSV)
DES exposure in utero
Immunosuppression therapy
Chronic irritation (e.g., from pessaries)
Irradiation for cervical disease
Possible association
HSV (may be a cofactor with HPV)
Previous hysterectomy for benign disease
Unknown association
Cigarette smoke
Other vaginal infections (e.g., cytomegalovirus)

appearances on gross examination; it can be papillary, growing in a cauliflower-like fashion, infiltrative or nodular, and ulcerative in type with raised borders and shallow ulcerated beds.

Most squamous carcinomas are composed of atypical and polymorphic tumor cells in different degrees of differentiation with dense eosinophilic cytoplasm and large nuclei with irregular chromatin distribution. Three histologic grades are recognized; well differentiated, moderately differentiated, and poorly differentiated. The tumors can also be classified as keratinizing if there is evidence of intracytoplasmic keratin or pearl formation and nonkeratinizing if this differentiation is lacking. Other varieties, such as spindle cell or verrucous carcinoma, are rare.

The fact that vaginal tumors resemble those in nearby structures is clinically significant, because frequently cancer at one site prefigures disease at the corresponding location. The link between tumors is so strong that many authors do not accept a vaginal tumor as primary if it occurs in a patient with history of cervical cancer. In general, it is accepted that patients with a previous history of cervical cancer should have a 5-year disease-free interval after treatment for invasive cancer and a 2-year interval between carcinoma in situ and the newly diagnosed vaginal lesion.⁴

Consequently, careful scrutiny and follow-up are necessary in patients with previous genital cancers. Extra vigilance is warranted even in women with conditions that are associated with cancer, such as cervical dysplasia or infection with human papillomavirus (HPV).

Predisposing factors

Nonviral. The literature cites numerous factors that may predispose women, particularly older women, to vaginal cancer (Table I). One suggested factor is chronic irritation. Chronic or irritant vaginitis is not frequent today, but it was commonly seen when pes-

saries were used as therapeutic devices for uterine prolapse. The vaginal mucosa shows marked hyperkeratosis or thickening, acanthosis, and inflammation. Pessaries left in place for long periods of time produce metaplastic and subsequent dysplastic alterations of the squamous mucosa. Today contact vaginitis is more likely caused by secondary chemical irritation and douches.

Previous hysterectomy for benign disease may also be a factor in primary vaginal cancer. In 1984 Bell et al.5 reported a 36% incidence of vaginal carcinoma in 87 women who had undergone hysterectomy for benign disease. Hoffman et al.6 subsequently found that 22 of 26 women with vaginal intraepithelial neoplasia had been so operated. Seven of the hysterectomies were for benign disease, whereas 15 were for cervical intraepithelial neoplasia or cancer. Two of the women in this series later had invasive squamous cell carcinoma of the vagina. More recently, however, Herman et al.7 have cast doubt on the association between hysterectomy and ensuing vaginal cancer. To investigate this relationship, they compared 49 patients with vaginal cancer with 49 control subjects matched for age, race, and a prior history of dysplasia or neoplasia. They concluded that hysterectomy was a very low risk factor for the development of vaginal cancer.7

A strong link also appears to exist between primary vaginal cancer and endometriosis. As is well known, ectopic endometrial tissue responds to hormonal stimulation and thus is capable of proliferating and giving rise to hyperplasias and carcinomas. A few cases have been reported in which ectopic tissue produced primary adenocarcinoma of the vagina, usually of the endometrioid type.⁸

Furthermore, clear cell adenocarcinoma of the vagina is well recognized in young women who were exposed in utero to the synthetic estrogen DES. The risk has recently been found to be higher for those patients whose mothers received DES before the twelfth week of gestation. This true primary vaginal neoplasm occurs in young women between 15 and 25 years of age, who frequently have associated vaginal adenosis, vaginal adhesions, or both. Many patients exposed to DES have an extensive transformation zone in the cervix and vagina, which on colposcopic evaluation may show mosaicism, punctuation, and areas of white epithelium representing squamous metaplasia. Benign glandular columnar epithelium is present in areas of adenosis, and whether benign adenosis evolves to carcinoma is still controversial. Carcinomas arising in DES-exposed patients may have several histologic patterns: tubulocystic, which is seen more frequently in older women and is associated with good 5-year survival, and solid,

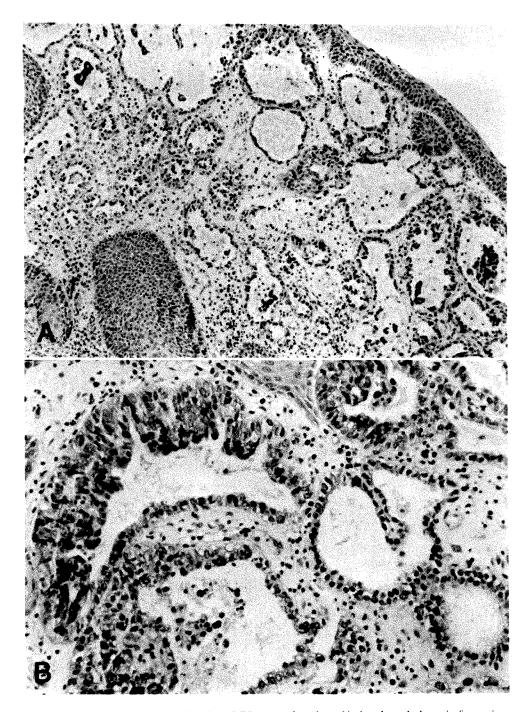


Fig. 1. A, Adenocarcinoma arising in a DES-exposed patient. Notice the tubulocystic formation. (Hematoxylin-eosin stain; ×200.) B, Cells with prominent nucleus lining the glandular structures. (Hematoxylin-eosin; magnification ×250.)

papillary, or mixed variants, which are seen in young girls and are associated with a poor prognosis. The tumors are composed of cells with clear cytoplasm and prominant round nucleus. Some cells may acquire a characteristic appearance with a large nucleus, prominant nucleolus, and scant cytoplasm (hobnail cells) (Fig. 1).9

Although relatively few DES-exposed women develop clear cell adenocarcinoma (1 per 1000 from birth through age 34 years),10 several factors apparently increase the risk of cancer in individual patients. These include a maternal history of prior miscarriage, exposure to DES in early gestation, birth in the fall, and prematurity.11 Given the low risk of clear cell adeno-

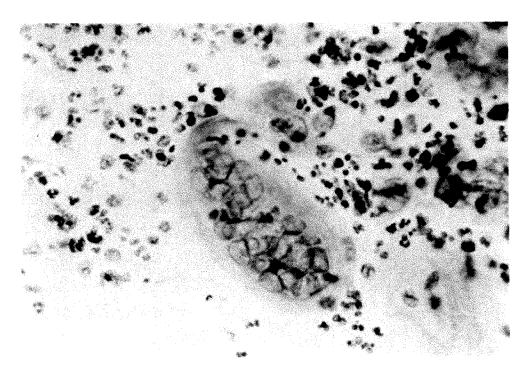


Fig. 2. Papanicolaou smear showing a multinucleated giant cell with washed-out nuclei, characteristic of herpes infection.

carcinoma, Melnick et al.¹² have postulated that DES is an incomplete carcinogen and that other cofactors may be involved in the pathogenesis of this disease. It now appears that many DES daughters who develop clear cell adenocarcinoma are also infected with HPV.

As with DES, the association between cervical cancer and subsequent vaginal cancer is strong. However, whether vaginal carcinoma represents a primary cancer or a late recurrence of the first disease is nearly impossible to know when the vaginal tumor arises 5 or more years after the cervical cancer. In a retrospective study of 763 women treated for invasive carcinoma of the cervix, Kapp et al. ¹³ found that patients were 14 times more likely to develop subsequent neoplasms involving the vagina or vulva than other body sites. Although it is very possible that the development of vaginal tumors merely reflects multiple neoplastic syndrome, it is interesting to note that most of the women in the study of Kapp et al. had been treated with ionizing radiation.

Many investigators believe that radiation therapy for cervical disease can induce vaginal cancer. It is well known that the amount of radiation reaching the vagina in such circumstances is not enough to kill cells but is sufficient to have a mutagenic effect. Estimates are that about 50% of patients who undergo irradiation may develop dysplasias in situ carcinomas.

In such patients the resultant vaginal cancer would be considered primary. Using a 5-year or more interval and cytologic smears to differentiate primary from secondary vaginal cancers, Murad et al.15 concluded that the risk of cancer may be greater after irradiation of a cervical carcinoma. In another study, Pride and Buchler¹⁶ found that women with cervical carcinoma ran a small but significant risk of radiation-induced cancer of the upper vagina 10 or more years after treatment.16-19 Okagaki et al.20 studied the deoxyribonucleic acid (DNA) content of cells in 60 patients identified as having dysplasias after radiation treatment. The cells were classified as diploid, aneuploid, or polyploid, and they correlated their findings with survival. Patients with aneuploid tumors had shorter survival times and earlier recurrences; thus they concluded that the presence of aneuploid cellular content was indicative of a poor prognosis.20

Of course, it is possible that primary vaginal tumors are really more common than estimated but that by the time of discovery cervical involvement leads most clinicians to assume that they are dealing with a primary cancer of the cervix. Nevertheless whether this is true, vaginal smears should be performed frequently in women who previously had irradiation for cervical cancer. In such patients flow cytometry may help determine the DNA population of the cells.

Vigilance may also be warranted in women who have undergone immunosuppression therapy, because in situ vaginal cancer has been found in organ-recipient patients and those patients with conditions such as

Fig. 3. Papanicolaou smear showing cellular changes consistent with HPV infection: perinuclear halos, enlarged nucleous, binucleation, and dense orangiophylic and granular cytoplasm.

chronic granulomatous disease.²¹ The associated tumors have occurred either as an isolated process or as part of the neoplastic syndrome of the lower genital tract. Immunosuppression is also believed to increase the risk of certain viral infections associated with genital cancers.

Viral. Viral infection, particularly with herpes simplex virus (HSV) or HPV, has been increasingly implicated in the pathogenesis of vaginal cancer. These infections follow the pattern of venereal diseases that can affect both men and women; except in rare cases, they do not appear before the onset of sexual activity.

HSV derives from a large class of DNA viruses that include varicella zoster, cytomegalovirus, and Epstein-Barr virus. After viral penetration of the human cell wall, the nucleocapsid is released, and the viral DNA enters the nucleus of the host cell. The dorsal spinal ganglia are believed to harbor latent viruses, which when activated travel down the axons and produce lesions in the skin supplied by the sensory neuron. Infections occurring above the waist are generally caused by HSV 1, whereas those occurring below the waist are generally caused by HSV 2; however, there is significant crossover between viral types in these two areas. Indeed, recent reports point to the increasing frequency with which HSV 1 is diagnosed as the cause of genital lesions.²²

In 1966 Naib et al.²³ reported an increase in the rate of cervical cancer in women with cytologically proven

herpetic cervicitis. Later, many seroepidemiologic studies, although not all, confirmed the prevalence of HSV 2 antibodies or antigens among women with cervical neoplasia. However, it has since been shown that when the data are controlled for sexuality, the association between these lesions and HSV 2 is not significant. Hence it is possible that these findings merely reflect an earlier age of first intercourse and multiple sexual partners. Moreover, no causal link has been established between HSV and genital cancers. Still, given the epidemiologic association, it is recommended that women with genital HSV infections be examined annually by Papanicolaou smear. HSV is readily identifiable in Papanicolaou smears as a multinucleated giant cell with a multiple, washed-out nuclei (Fig. 2).

In recent years attention has shifted from HSV to HPV as an etiologic factor in the development of gynecologic cancers, particularly in younger women. Several lines of evidence—epidemiologic, clinical and experimental—support the association.

With its icosahedral virion capsids and circular double-stranded DNA, HPV forms a subgroup of the papovavirus family. More than 50 distinct types of HPV exist, 12 of which are associated with lesions of the anogenital tracts. Types 6 and 11 are often found in condyloma acuminata or low-grade lesions. ²⁶ Types 16, 18, 31, 33, 35, 39, 45, and 52 have been linked to anogenital cancers²⁷; types 16 and 18 have been closely associated with squamous cell carcinomas. ^{26, 28} In ma-



Fig. 4. Condylomatous vaginitis. The squamous mucosa is thickened, and koilocytes can be identified in the surface epithelium. (Hematoxylin-eosin; magnification $\times 150$.)

lignant transformation, the virus becomes integrated into the host cell genome. As a result, abnormalities of host cell DNA replication arise, and a marked tendency toward aneuploidy develops. Animal studies show that the rate of malignant conversion depends on the viral strain, host species, and individual response.

HPV infections of the anogenital area have increased dramatically during the past 2 decades; the estimated incidence is three times that of genital herpes in the United States.²⁹ Studies have shown that women with a history of these infections are four times more likely to develop cervical carcinoma in situ than their uninfected counterparts.³⁰ The natural history of the progression to intraepithelial neoplasias is best understood in cervical lesions.^{31,35} Whether a lesion regresses, remains the same, or progresses depends on various factors, such as the histologic grade of the tumor and the HPV type. With highgrade lesions, there is almost total loss of squamous maturation and a higher risk of progression to invasive cancer.³³

Although infection with HPV appears necessary for the development of malignancy, some authors argue for the involvement of additional environmental or host-related cofactors.^{27, 31, 36} Popescu and DiPaolo³⁶ have proposed that HPV integration on the mammalian genome increases genetic instability and uncontrolled cell division, thereby making the cell more sus-

ceptible to cofactors that complete neoplastic conversion. A case has also been made for a possible link between HSV and HPV in the development of anogenital cancers.³⁷

That viruses may serve as cofactors in the pathogenesis of cervical cancer was demonstrated in a prospective study of 950 DES daughters by Adams et al.²² They found that HSV 1 antibody levels were significantly higher in the 23 women who developed cervical intraepithelial neoplasia during the study than in a well-matched control population. They also found that 73 of patients with DES who were negative for HPV when the study began became infected by its end. These findings suggest that HSV 2 infection may precede HPV infection and that the two may be essential to inducing squamous cell carcinoma. Certainly, the concept of cofactors is intriguing, because HPV alone can be found in a significant percentage of women with cytologically normal results.^{29, 32}

Various gradations of dysplasias are associated with HPV lesions (Figs. 3 through 6). The hallmark of condylomatous lesion is the presence of the koilocyte, which is a hyperchromatic, wrinkled irregular nucleus surrounded by abundant clear cytoplasm. The squamous mucosa shows various changes, such as acanthosis, papillomatosis, elongation of the rete pegs, and hyperkeratosis with or without parakeratosis. Associated dermal inflammation is also noted. The dys-

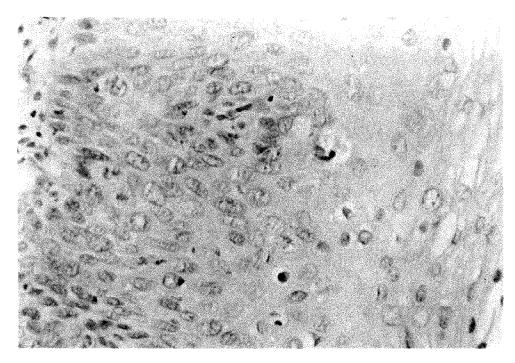


Fig. 5. Moderate dysplasia associated with HPV infection. Notice the lack of maturation of the basal cells, approaching about half the thickness of the squamous mucosa. (Hematoxylin-eosin; magnification $\times 250$.)

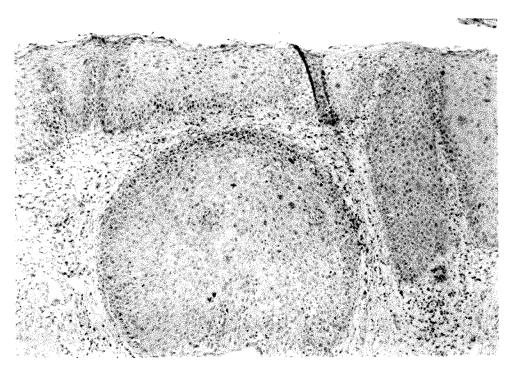


Fig. 6. Severe dysplasia in association with condylomatous changes. (Hematoxylin-eosin; magnification \times 150.)

plastic changes are similar to those that occur in the cervix.

Prognosis

The prognosis of vaginal cancer is highly dependent on the stage of the disease. The International Federation of Gynecologists and Obstetricians classifies vaginal cancer as follows:

Stage 1: Squamous carcinoma limited to the vaginal wall

Stage II: Mural extension without involvement of the pelvic side walls

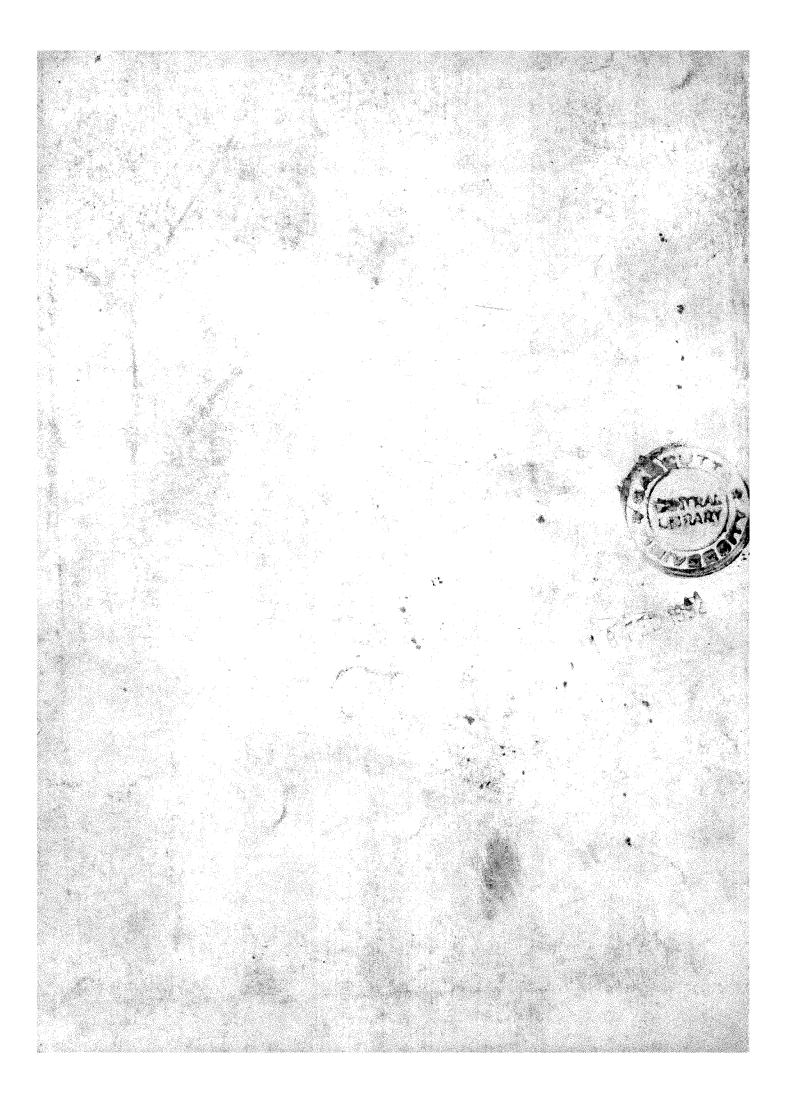
Stage III: Involvement of one or both pelvic side walls Stage IV: Tumor beyond the pelvis or involvement of the bladder or rectal mucosa

In general, the 5-year survival rate for patients with Stage I disease is approximately 80% to 90%, 50% for Stage II, and 20% for advanced Stages III and IV. Surgery and radiation therapy, either alone or in combination, are the preferred modalities of treatment.

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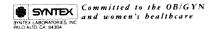
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Carcinogenesis: Animal studies have not been done

Mutagenesis: Mutagenicity studies were negative

Impairment of Fertility: Animal studies showed no impairment of fertility

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

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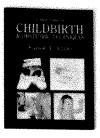
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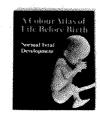
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Contrainflications — OCs should not be used in women with any of the following: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Endometrial carcinoma or other known or suspected stronger-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contra-ceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

should be strongly advised not to smoke.

Use of OCs is associated with increased risks of serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although risk of serious morbidity/
mortality is very small in heatthy women without underlying risk factors. Morbidity/mortality risk increases
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prescribing OCs should be familiar with the following information relating to these risks, This information is
based principally on data involving OCs with higher doses of estrogen and progestogen than those commonly
used today. Effect of long-term use of lower estrogen and progestogen formulations is yet to be determined.)

1. Thromboembolic Disorders and Other Vascular Problems — MYDCARDIAL. INFARCTION (M), An increased
risk of MI has been attributed to OC use. Risk is primarily in smokers or women with other underlying risk factors
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heart attack for current OC users is estimated to be two to six risk is very low under the age of 30.
Smoking combined with OC use contributes substantially to incidence of MIs in women in their mid-thirties or
older with smoking accounting for majority of excess cases. Mortality rates associated with circulatory disease
increase substantially in smokers over the age of 35 and nonsmokers over the age of 40 among OC users.
OCs may compound effects of well-known insk factors. Such as hypertension, diabetes, hyperlipidemias, age and
obesity in particular some progestogens decrease HDL cholesterol and cause glucose intolerance, while
estrogens may create a state of hyperinsulinism. OCs have been shown to increase blood pressure among users
(see Warnings). Similar effects on risk factors are associated with increased risk of heart disease. Use OCs with
caution in women with cardiovascular diseas

due to OCs is not related to length of use and disappears after pill use is stopped.

A 2- to 4-fold increase in relative risk of postoperative thrombosin or conditions has been reported with OCs. Relative risk of venous thrombosis in women with predisposing conditions is twice that of women without such conditions. If feasible, discontinue OCs at least 4 weeks prior to and for 2 weeks after elective surgery of a type associated with increased risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is associated with an increased thromboembolic isk, start OCs no earlier than 4 to 6 weeks after delivery in women not breast-feeding, or a mid-timester pregnancy termination. CFREBROVASCULAR DISEASES. OCs increase relative and attributable risks of creatrovascular events (thrombotic and hemorrhagic strokes); in general, risk is greatest among older (> 35 years), hypertensive women who smoke, hypertension is a risk factor for users and nonusers, for both types of strokes, while smoking interacts to increase hemorrhagic stroke risk.

DOSE-RELATED RISK OF VASCULAR DISEASE FROM OCS. A positive association has been observed between amount of estrogen and progestogen in OCs and vascular disease isk. A decline in serum high density ilipoproteins (HDL) is reported with many progestational agents. Serum HDL decline is associated with increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, net effect depends on balance archieved between doses of estrogen and progestogen and nature and absolute amount of progestogen regimen prescribed should contain the least amount of estrogen and progestogen compatible with a

The dosage regimen prescribed should contain the least amount of estrogen and progestogen compatible with a low failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of

low failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of estrogen.

PERSISTENCE OF RISK OF VASCULAR DISEASE. Two studies have shown persistence of vascular disease risk for ever-users of OCs. In a U.S. study, Mil risk after OC discontinuation persists for at least 9 years in women 40-49 years who had used OCs for five or more years; increased risk was not demonstrated in other age groups. In a study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after OCs stopped, although excess risk was very small. Both studies used OC formulations with 50 micrograms or higher of estronges.

of estrogens.

2. Estimates of Mortality from Contraceptive Use — A study using data from several sources concluded that with the exception of OC users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The possibility of increased mortality risk with age for OC users is based on data from the 1970s — but reported in 1983. However, current practice involves use of lower estrogen dose formulations combined with careful restriction of OC use to women without the various risk factors listed in this labeling.

the various risk factors listed in this labeling. Changes in practice and new data suggesting that cardiovascular disease risk with OCs may be less than previously observed prompted the Fertility and Maternal Health Drugs Advisory Committee to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with OC use after age 40 in healthy nonsmokers leven with newer low-dose formulations), greater potential health risks are associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if effective, acceptable contraception is not available.

The Committee concluded that the benefits of OC use by healthy nonsmoking women over 40 may outweigh the possible risks. Older women, as all women who take OCs, should use the lowest possible effective dose

formulation.

3. Carcinoma of the Reproductive Organs — Numerous epidemiological studies have looked at the incidence of breast, endometrial, ovarian and cervical cancer in women using OCs, Overwhelming evidence suggests that OC use is not associated with an increase in risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. A few studies show a slightly increased relative risk of developing breast cancer, although the methodology of these studies, including differences in examination of users and nonusers, and in age at start of use, has been questioned.

Some studies suggest that OC use is associated with an increased risk of cervical intraepithelial neoplasia in some populations of women. However, controversy continues about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between OC use and breast and cervical cancers, a cause and effect relationship has not been established.

4. Hepatic Neoplasia—Benign hepatic adenomas are associated with OC use, although incidence is rare in the U.S. Indirect calculations estimate attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

British studies have shown an increased risk of hepatocellular carcinoma in long-term (> 8 years) OC users; these cancers are extremely rare in the U.S. and attributable risk (excess incidence) of liver cancers in OC users approaches less than one per million users.

5. Ocular Lesions — There are clinical case-reports of retinal thrombosis with OC use. Discontinue OCs if there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions; undertake appropriate diagnostic and therapeutic measures immediately.

6. Oral-Contraceptive Use Believe or During Farty Pregnancy—Extensive epidemiological studies revealed no increased risk of birth defects when OCs used prior to pregnancy. Studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. Oc-induced withdrawal bleeding should not be used as a pregnancy test. Do not use OCs during pregnancy to treat threatened or habitual abortion. Rule out pregnancy if two consecutive periods missed before continuing OC use. If patient has not adhered to prescribed schedule, consider pregnancy at time of first missed period. Discontinue OC if pregnancy confirmed.

7. Gallbladder Discase—Earlier studies reported an increased lifetime relative risk of gallbladder surgery in users of OCs and estrogens, more recent studies show that the relative risk of developing gallbladder disease among OC users may be minimal, which may be related to use of formulations with lower hormonal estrogen and progestogen doses.

In Carbonylaride and Lipid Metabolic Effects — OCs cause glucose intolerance in a significant percentage of users. OCs with greater than 75 µg of estrogen cause hyperinsulinism; lower estrogen doses cause less glucos intolerance. Progestogens increase insulin secretion and create insulin resistance (effect varies with different agents). Observe prediabetic and diabetic women carefully while taking OCs. In non-diabetic women, OCs have

no apparent effect on fasting blood glucose.

A small proportion of women will have persistent hypertriglyceridemia while on OCs. Changes in serun triglycerides and lipoprotein levels have been reported in OC users (see Warnings).

regiscences and ipportern levels nave been reported in OC users (see warnings).

9. Elevated Blood Pressure — Increase in blood pressure has been reported in women on OCs, increase is more likely in older OC users and with continued use. Data show that incidence of hypertension increases with increasing quantities of progestogens.

Encourage women with history of hypertension or hypertension-related diseases, or renal disease to use another contraceptive method. Monitor hypertensive women electing to use OCs closely, discontinue OC if significant blood pressure elevation occurs. For most women, elevated blood pressure returns to normal after OC stopped. No difference in occurrence of hypertension among ever- and never-users exists.

Headache — Discontinue OC and evaluate cause at onset or exacerbation of migraine, or if new pattern of

10. Headacene—Discommine U.C. prevision evaluate cause at onset or exacerbation or migraine, or it new pattern of headache (i.e. recurrent, persistent, severel develops.

11. Elevering Irregularities — Breakthrough bleeding and spotting sometimes occur especially during first 3 months of use. Type and dose of progestogen may be important. Consider non-hormonal causes and take adequate diagnostic measures to rule our malignancy or pregnancy in event of breakthrough bleeding, as with any abnormal vaginal bleeding. If pathology excluded, time or a formulation change may solve the problem. In the event of amenometra, rule our pregnancy, Some women encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

Precautions

respectanty when such a contintion was pre-existent.

1. Physical Examination and Follow Up — A complete medical history and physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, conduct appropriate diagnostic measures to rule out malignancy Monitor women with strong lamily history of breast cancer or who have treast nodules with particular care. 2. Lipid Disorders — Follow women being treated for hyperlipidemias closely if they elect to use OCs. Some progestogens may elevate LDL levels and may render control of hyperlipidemias more difficult. (See Warnings) 3. Liver Function — Discontinue OC if jaunctice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. Fluid Retention — OCs may cause some degree of fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions possibly aggravated by fluid retention. 5. Emotional Disorders — if significant depression occurs stop medication and use alternate contraceptive method in attempts to determine if symptom is drug related. Observe carefully those with history of depression and stop drug if depression recurs to serious degree. 6. Contact Lenses — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities are associated with concomitant rifampin use. A similar association though less marked, is suggested with barbiturates, pnerylbutazone, phenylbutazone, phenyl and blood components may be affected by OCs: a Increased prothrombin and factors VII, VIII, X, and X, decreased antithrombin 3: increased noreprephrine-induced platelet aggregability b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), 14 by column or by radioimmunoassay Free T3 resin uptake is decreased, reflecting the elevated TBG, free T3 resin uptake is decreased, reflecting the elevated TBG, free T3 resin uptake is decreased, reflecting the elevated TBG, free T3 resin uptake is decreased. reflecting the elevated TBG, free T3 resin uptake is decreased. reflecting the elevated TBG, free T3 resin uptake is decreased. In Calcose to the case of the concentration of the contraction of the contr

Information for the Patient — See Patient Package Labeling.

Adverse Reactions — An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, arreial thromboembolism; pulmonary embolism; myocardial infarction; cerebral hemorrhage; cerebral thrombosis; hypertension, gallbladder disease; hepatic adenomas or benign

There is evidence of an association between the following conditions and OC use, although additional confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis.

There is evidence of an association between the following conditions and OC use, although additional confirmatory studies are needed mesenteric thrombosis; retinal thrombosis; retinal thrombosis; studies are needed mesenteric thrombosis; retinal thrombosis; retinal thrombosis; studies are needed mesenteric thrombosis; retinal thrombosis; retinal thrombosis; studies are believed to be drug-related nausea; vornifing, gastrointestinal symptoms (such as abdominal campas and bloating); breakthrough bleeding; spotting; change in mensitual flow, amenorinea; temporary intertility after treatment discontinued; edema: melasma which may persist; breast changes tendemess, enlargement, secretion; change in weight (increase or decrease); change in mensitual ensoin and secretion; diminitudin in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidassis, change in corneal curvature (steepering); intolerance to contact tenses.

The following adverser exactions have been reported in OC users and the association is neither confirmed nor refuted; congenital anomalies; premenstrual syndrome, cataracts; optic neuritis; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hissuitism; loss of scalp hair, eythema multiforme; eythema nodosum; hemorrhagic eruption; vaginitis; porphyria; impaired renal function, hemolytic uremic syndrome; budd-Chari syndrome; ache; changes in libido; collis; sickle-cell disease; cerebral-vascular disease with mitral valve prolasse; hypus-like syndromes.

Overdosage — Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nause, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits — The following noncontraceptive health benefits related to OC use are supported by epidemiological studies that largely utilized OC formulations containing doses exceeding 0.035 mg of ethingle

Dosage and Administration — For maximum contraceptive effectiveness, take TRIPHASIL* (levonorgestrel and ethinyl estradiol tablets — triphasic regimen 21- and 28-day regimens) exactly as directed and at intervals not over 24 hours.

(If TRIPHASIL.* is first taken later than first day of first menstrual cycle of medication or postpartum, contra-ceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.) For full details on dosage and administration see prescribing information in package insert.



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November Part 1

CLINICAL SECTION

Clinical Opinion

An epidemic of antiabortion violence in the United States

1263

David A. Grimes, MD, Jacqueline D. Forrest, PhD, Alice L. Kirkman, JD, and Barbara Radford, MA Los Angeles, California, New York, New York, and Washington, D.C.

From 1977 to 1988, 110 cases of antiabortion violence took place in the United States.

Multifetal pregnancy reduction and disposal of untransplanted embryos in contemporary Jewish law and ethics

1268

Richard V. Grazi, MD, and Joel B. Wolowelsky, PhD Brooklyn, New York

Jewish law and ethics regarding multifetal pregnancy reduction and disposal of untransplanted embryos are presented and contrasted with those of other religious and secular institutions.

Clinical Articles

Should anticardiolipin tests be performed in otherwise healthy pregnant women?

1272

E. Nigel Harris, MPhil, MD, DM, and Joseph A. Spinnato, MD Louisville, Kentucky

In healthy pregnant women anticardiolipin test results are infrequently positive, are not associated with pregnancy complications, and occur at lower levels than in the antiphospholipid syndrome.

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The effect of maternal cocaine use on the fetus: Changes in antepartum fetal heart rate tracings

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Bannie L. Tabor, MD, Alex R. Soffici, MD, Toni Smith-Wallace, LVN, and M. Lynn Yonekura, MD *Torrance, California*

Recent maternal cocaine use causes changes in antepartum fetal heart rate tracings suggestive of alterations in fetal behavioral states.

Qualitative evaluation of uterine contractions recorded by a double guard-ring tocodynamometer

1282

Masahiro Shinmoto, MD, Tatsuhiko Kawarabayashi, MD, Masahiko Ikeda, MD, and Hajime Sugimori, MD Saga, Japan

Qualitative characteristics of uterine contractions in various labors were evaluated by a small double guard-ring tocodynamometer.

A prospective, randomized comparison of the Pipelle endometrial sampling device with the Novak curette

1287

Thomas G. Stovall, MD, Frank W. Ling, MD, and Patrick L. Morgan, MD Memphis, Tennessee

This prospective trial confirms that endometrial sampling with a Pipelle device is effective and is less painful than that with the Novak curette.

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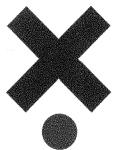
Cefotan arithmetic.

7.299

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less frequent dosing than cefoxitin^{3,4}



several times the half-life of cefoxitin^{5,6}



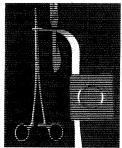
single-dose prophylaxis/ twice-daily treatment



CEFOTAN (cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: 1. Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. Am J Surg. 1988; 155(5A):101-102. 2. Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. Am J Surg. 1988; 155(5A):96-101. 3. CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. 4. Physicians' Desk Reference. ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp. 1355-1357. 5. Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. Infect Surg. April 1986 (suppl), pp. 11-14. 6. Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PI, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. Am J Surg. 1988;155(5A):67-70. 7. Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. MMWR. 1989;38(suppl S-8):1-43.



Count on EFOTAN[®]IM/IV (cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use (FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.)

INDICATIONS AND USAGE

Treatment: CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Treat_Infections_caused by F.ord. // kebsiella species (including // pneumoniae), Proteus mirabilis, and Proteus sp (which may include the organisms now called Proteus vulgaris, Providencia content, and Monagorella morecarii).

mirabilis, and Proteuts sp (which may include the organisms flow called Proteus Volgans, Providenta, rettgeri, and Morganella morganii).

Lower Respiratory Tract Infections caused by Streptococcus pneumoniae (formerly D pneumoniae) Staphylococcus aureus (penicillinase- and nonpenicillinase- producing strains), Haemophilus influen (including ampicillin-resistant strains), Klebsiella species (including K pneumoniae), E coli, Proteus

Staphylococcus aireus (pericillinase- and nonpenicillinase-producing strains), Haemophilus influenzae (including ampicillin-resistant strains), Klebsiella species (including K pneumoniae), E coli, Proteus mirabilis, and Serratia marcescens:

Skin and Skin Structure Infections caused by Staphylococcus aureus (penicillinase- and nonpenicillinase-producing strains), Staphylococcus epidermidis, Streptococcus progenes, Streptococcus species (excluding enterococci), E coli, Klebsiella pneumoniae, and Peptococcus and Peptostreptococcus species (excluding enterococci), E coli, Klebsiella peneumoniae, and Peptococcus and Peptostreptococcus species (excluding penicillinase-producing strains), Staphylococcus epidermidis, Streptococcus species (excluding enterococci), group B streptococcus and Peptostreptococcus species) and B thetaiotaomicron, Flobacterium species, and gram-positive anaerobic cocci (including Peptococcus and Peptostreptococcus species).

Intra-abdominal Infections caused by E coli, Klebsiella species (including K pneumoniae), Streptococcus species (excluding enterococci), Bacteroides species (excluding B distasonis, B ovatus, and B thetaiotaomicron), and Clostridium species.*

Bone and Joint Infections caused by Staphylococcus aureus.*

Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known, however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Detote the aminoglycoside are used concomitantly with an aminoglycoside bacteria. The dosage recomm

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics. WARNINGS

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoletan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures. Pseudomembranous colitists has been reported with the use of cephalosporins (and other broadspectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by Clostridium difficile is one primary cause of antibiotic associated colitis.

biotic-associated colitis

antibiotic-associated colitis.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by C difficile. Other causes should also be considered. In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated. PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested

flushing, sweating, headache, and tackycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an appropriate process.

Drug/Laboratory Test Interactions: A false positive reaction for glucose in urine may occur with Benedicts or Fehling's solution.

As with other cephalosporins, high concentrations of celotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of

creatinine reported.

Carcinogenesis, Mutagenesis, impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of

500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylintotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Usage In Pregnancy: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE FRACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

ADVENSE REAGINANS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy,
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and

Gastrointestinal symptoms occurred in 1.3% or patients, the most request that distinct characters are marked (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching the results of the state of the stat

(1 in 700).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

During postmarketing experience with CEFOTAN, agranulocytosis, anaphylactic reactions, fever, hemolytic anemia, leukopenia, prolonged prothrombin time with or without bleeding, pseudomembranous colitis, and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

Treatment: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intra-muscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be deter-mined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN			
Type of Infection	Daily Dose	Frequency and Route	
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM	
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM	
Severe	4 grams	2 g every 12 hours IV	
Life-Threatening	6* grams	3 a every 12 hours IV	

^{*}Maximum daily dosage should not exceed 6 grams.

Prophylaxis: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

Impaired Renal Function: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION			
mL/min	Dose	Frequency	
>30	Usual Recommended Dosage*	Every 12 hours	
10-30	Usual Recommended Dosage*	Every 24 hours	
<10	Usual Recommended Dosage*	Every 48 hours	

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min. When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

Weight (kg) × (140 - age) Males 72 × serum creatinine (mg/100 mL)

Females:

 $0.9 \times \text{value for males}$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

Manufactured for



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Spontaneous preterm birth: A case-control study

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Ine de Haas, MD, Bernard L. Harlow, PhD, Daniel W. Cramer, MD, ScD, and Fredric D. Frigoletto, Jr., MD Boston, Massachusetts

Demographic characteristics and the influence of smoking, weight, reproductive history, and maternal diethylstilbestrol exposure were evaluated with respect to the risk of spontaneous preterm delivery.

An extraordinarily high CA 125 level in a woman without apparent pathologic foci of CA 125 production: Dissociation between serum levels of CA 125 and CA 130

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Fuminori Kobayashi, MD, Shingo Fujii, MD, Hirofumi Nonogaki, MD, Yoshihiko Nanbu, MD, Toshiko Iwai, MD, Ikuo Konishi, MD, Norimasa Sagawa, MD, Takahide Mori, MD, Masako N. Hosono, MD, and Keigo Endo, MD

Kyoto, Japan

A case of extraordinarily high serum levels of CA 125 without the apparent pathologic foci of CA 125 production is presented.

Severe obstructive sleep apnea and associated snoring documented during external tocography

1300

David M. Sherer, MD, Christine B. Caverly, RN, and Jacques S. Abramowicz, MD Rochester, New York

Snoring after apneic episodes documented by external tocography in the third trimester subsequently led to diagnosis of severe obstructive sleep apnea.

Management of fetal hemolytic disease by cordocentesis. II. Outcome of treatment

1302

Carl P. Weiner, MD, Roger A. Williamson, MD, Katharine D. Wenstrom, MD, Susan L. Sipes, MD, John A. Widness, MD, Stanley S. Grant, RN, and Louise Estle, RN *Iowa City, Iowa*

Simple intravascular fetal transfusion safely suppresses fetal erythropoiesis and allows term delivery.

Lack of an association between late fetal death and antiphospholipid antibody measurements in the second trimester

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James E. Haddow, MD, Neal S. Rote, PhD, Dorene Dostal-Johnson, MS, Glenn E. Palomaki, BS, Andrea J. Pulkkinen, MS, and George J. Knight, PhD Scarborough, Maine

Antiphospholipid antibody measurements obtained at ≥ 15 weeks' gestation are not helpful in identifying pregnancies at risk for fetal death.

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Cord Blood pH...adding objectivity the newborn assessment.

Corometrics 220 pH System provides a simple, immediate and objective means of obtaining cord blo

Umbilical cord blood sampling provides a reliable measurement for objectively ing the presence or extent of fetal asphyxia at delivery. Assessment of cord blood j is indicated when ominous electronic fetal heart rate patterns are present, when oth intrapartum distress are observed, or when unsuspected neonatal distress is noted. In conjunction with traditional APGAR scoring methods and the assessment of fetal patterns, cord blood pH can serve as a more precise indicator of newborn status after as compared to any single assessment method. Cord blood pH values can also retrospectively evaluate intrapartum labor management practices.

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 - The disposable pH cartridge is inserted into the 220 pH monitor.
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Umbilical cord blood samplin, Corometrics 220 pH System . . pH assessment system needs delivery room. Call or wri system demonstration of our color procedu

1. Page et al, Correlation of Programs (extens with Applic Profesand fetal heart rate trikings, 200) on the Profesor, 1986; 156-1506

2. Yeomans et al, Unadical Construction of Description of the uncomplicated term value of the United States of the



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Severe ovarian hyperstimulation in a spontaneous singleton pregnancy

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Gregory F. Rosen, MD, MS, and Mitchell W. Lew, MD

Irvine and Long Beach, California

Severe ovarian hyperstimulation syndrome accompanying a spontaneous but otherwise normal singleton pregnancy is reported.

Pseudoprognathism—An auxiliary ultrasonographic sign for transvaginal ultrasonographic diagnosis of cleft lip and palate in the early second trimester

1314

M. Bronshtein, MD, N. Mashiah, MD, I. Blumenfeld, DMD, and Z. Blumenfeld, MD Haifa, Israel

Transvaginal ultrasonographic examination was helpful in correctly diagnosing three cases of cleft lip, by means of the pseudoprognathism sign on sagittal paramedial section of the fetal face.

Extended longitudinal study of uterine activity among low-risk women

1317

Denise M. Main, MD, Jeane A. Grisso, MD, Tiazu Wold, BA, Ellen Sim Snyder, MS, John Holmes, MS, and Grace Chiu, PhD

Philadelphia, Pennsylvania

This study demonstrates a significant association between prelabor contraction frequency and gestational age, time of day, and maternal weight.

Videolaseroscopy for oophorectomy

1323

Farr Nezhat, MD, Camran Nezhat, MD, and Sheryl L. Silfen, MD Atlanta, Georgia

Videolaseroscopic oophorectomy is a safe alternative to laparotomy for certain cases of removal of the ovaries.

Inappropriate secretion of antidiuretic hormone in Sheehan's syndrome: A rare cause of postpartum hyponatremia

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Chaim Putterman, MD, Yaniv Almog, MD, Yoseph Caraco, MD, David J. Gross, MD, and Eldad Ben-Chetrit, MD

Jerusalem, Israel

A patient with inappropriate antidiuretic hormone secretion and severe hyponatremia as the initial manifestation of early Sheehan's syndrome is described.

Congenital heart block: Successful prophylactic treatment with intravenous gamma globulin and corticosteroid therapy

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Risto Kaaja, MD, Heikki Julkunen, MD, Pirkko Ämmälä, MD, Anna-Maija Teppo, MSc, and Pekka Kurki, MD Helsinki, Finland

A mother with anti-Ro antibodies whose previous pregnancy ended in delivery of a child with congenital heart block was successfully treated with intravenous gamma globulin and corticosteroids.

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To be continued...



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†Number of patients continuing the method for 12 consecutive months; data on file, Ortho Pharmaceutical Corporation.

‡Data on file, Ortho Pharmaceutical Corporation. Consult the patient brochure for information on toxic shock syndrome (TSS) and wearing time.



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Amniotic fluid contains tissue factor, a potent initiator of coagulation

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Charles J. Lockwood, MD, Ronald Bach, PhD, Arabinda Guha, PhD, Xiaodong Zhou, MD,

Wayne A. Miller, MD, and Yale Nemerson, MD

New York, New York, and Lexington, Massachusetts

Amniotic fluid contains high concentrations of intact, partially active tissue factor, which increase with gestational age and account for the procoagulant property of amniotic fluid.

Laser vaporization of grade 3 vaginal intraepithelial neoplasia

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Mitchel S. Hoffman, MD, William S. Roberts, MD, James P. LaPolla, MD, James V. Fiorica, MD, and Denis Cavanagh, MD

Tampa, Florida

Laser vaporization was not efficacious treatment for grade 3 vaginal intraepithelial neoplasia diagnosed in the region of a vaginal cuff scar.

On reducing the frequency of severe abruptio placentae

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Jack A. Pritchard, MD, F. Gary Cunningham, MD, Signe A. Pritchard, RN, and Ruble A. Mason, RMT Dallas, Texas

The elimination of very high parity and the influx of large numbers of Latin American patients decreased the incidence of severe abruptio placentae by one half.

A rapid visual test for predicting fetal lung maturity

1351

Anthony J. Sbarra, PhD, Anjan Chaudhury, MD, Curtis L. Cetrulo, MD, Robert Mittendorf, MD, Chris Shakr, MD, Robert Kennison, MD, Johannes Jones, MD, and Joseph Kennedy, Jr., MD Boston, Massachusetts

A rapid bedside test that predicts when the usual indices for fetal pulmonary maturity will be positive or negative is proposed.

Fetal abdominoscrotal hydrocele

1353

P. Sasidharan, MD, Stanley Crankson, MB, and Saeed Ahmed, MB Riyadh, Saudi Arabia

Antenatal ultrasonography detected a fetal intraabdominal cyst that was an abdominoscrotal hydrocele, the neonatal diagnosis and management of which are described.

Increased serum levels of macrophage colony-stimulating factor in ovarian cancer

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F.-J. Xu, MD, S. Ramakrishnan, PhD, L. Daly, J.T. Soper, MD, A. Berchuck, MD, D. Clarke-Pearson, MD, and R.C. Bast, Jr., MD

Durham, North Carolina, and Minneapolis, Minnesota

Elevated serum levels of macrophage colony-stimulating factor that were observed in ovarian cancer in the absence of concomitant serum CA 125 elevation indicate its value as a complementary tumor marker.

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Jerome S. Kalur, JD, James N. Martin, Jr., MD, Kent A. Kirchner, MD, and John C. Morrison, MD Cleveland, Ohio, and Jackson, Mississippi

A rare complication of preeclampsia is sudden severe hypotension followed by hyponatremia and death.

Renal agenesis in association with malformation of the female genital tract

1368

Pedro Acién, MD, José A. Ruiz, MD, José F. Hernandez, MD, Francisco Susarte, MD, and Angel Martin del Moral, MD

Alicante, Spain

Renal agenesis is associated with ipsilateral blind vagina or agenesis of all organs derived from the urogenital ridge.

The association between fetal karyotype and mean corpuscular volume

1371

Susan L. Sipes, MD, Carl P. Weiner, MD, Katharine D. Wenstrom, MD, Roger A. Williamson, MD, and Stanley S. Grant, RN

Iowa City, Iowa

An elevated fetal mean corpuscular volume may be an indication for karyotypic evaluation.

Cytologic localization of epidermal growth factor and its receptor in developing human placenta varies over the course of pregnancy

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Cecilia A. Ladines-Llave, MD, Takeshi Maruo, MD, Augusto S. Manalo, MD, and Matsuto Mochizuki, MD Kobe, Japan, and Manila, Philippines

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1383

Nicole E. Menegakis, MD, and Marvin S. Amstey, MD Rochester, New York

A 37-year-old woman, who had normal coronary arteries, had a myocardial infarction attributed to smoking and Pitocin while in labor.

Endocrine effects in female weight lifters who self-administer testosterone and anabolic steroids

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William B. Malarkey, MD, Richard H. Strauss, MD, Daniel J. Leizman, MD, Mariah Liggett, PhD, and Laurence M. Demers, PhD

Columbus, Ohio, and Hershey, Pennsylvania

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Houston, Texas	
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Franceville, Gabon, and Clamart and Paris, France

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Lisa I. Yang, MD, Elizabeth S. Panke, MD, PhD, Phyllis A. Leist, PhD, Richard J. Fry, MD, and Richard F. Lee, PhD

Cincinnati. Ohio

The deoxyribonucleic acid probe test (PACE 2, Gen-Probe, San Diego) is equivalent to standard tissue culture in sensitivity, specificity, and positive and negative predictive values.

Loxoscelism threatening pregnancy: Five cases

1454

Philip C. Anderson, MD Columbia, Missouri

Bites of the most poisonous North American spider, Loxosceles reclusa, during pregnancy seem to cause no unusual risks to the fetus in this (first) report of five cases.

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1457

Takeshi Kumazawa, MS, Osamu Suzuki, MD, Hiroshi Seno, MD, Shigehiko Mizutani, MD, and Takatoshi Matsumoto, MD

Hamamatsu and Nagoya, Japan

Polyamine oxidase activity in pregnancy serum was steady at very low levels and not higher than that in nonpregnancy serum.

BASIC SCIENCE SECTION

Amnioinfusion increases amniotic pressure in pregnant sheep but does not alter fetal acid-base status

1459

Nicholas M. Fisk, MB, Dino A. Giussani, BSc, Michael J. Parkes, DPhil, Peter J. Moore, PhD, and Mark A. Hanson, DPhil

Reading, Berkshire, United Kingdom

Amnioinfusion of 5 to 15 L in seven ewes increased amniotic pressure but produced no change in mean fetal pH, PCO₂, PO₂, arterial pressure, or heart rate.

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David R. Powers, MD, and Robert A. Brace, PhD San Diego, California

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Oxytocin secretion and human parturition: Pulse frequency and duration increase during spontaneous labor in women

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Anna-Riitta Fuchs, DSc, Roberto Romero, MD, David Keefe, MD, Manuel Parra, MD, Enrique Oyarzun, MD, and Ernesto Behnke, MD

New York, New York, New Haven, Connecticut, and Santiago, Chile

In parturient women oxytocin is secreted in discrete pulses, the frequency and duration of which are significantly increased in the first, second, and third stages of spontaneous labor.

Dose-dependent effects of angiotensin II on the ovine fetal cardiovascular system

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Oliver W. Jones III, MD, Cecilia Y. Cheung, PhD, and Robert A. Brace, PhD San Diego, California

Angiotensin II affects ovine fetal arterial pressure, venous pressure, heart rate, and blood volume in a dose-dependent manner, suggesting that angiotensin II is an important regulator of the entire fetal cardiovascular system.

In vivo and in vitro effects of magnesium sulfate in the cerebrovascular bed of the goat

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Alfredo J. Perales, MD, Germán Torregrosa, PhD, Juan B. Salom, PhD, Francisco J. Miranda, MD, José A. Alabadí, BSc, Javier Monleón, MD, and Enrique Alborch, MD *Valencia, Spain*

Magnesium sulfate injected directly into the cerebral circulation or infused intravenously increases cerebral blood flow in conscious nonpregnant goats and relaxes isolated goat middle cerebral arteries.

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F.J. Montz, MD, J. McCabe Fowler, MD, A. John Wolff, BS, Silas M. Lacey, and Margie Mohler, PhD Los Angeles, California

Intraperitoneal recombinant tissue plasminogen activator was effective at preventing post-radical pelvic surgery adhesions in a canine model.

Effects of magnesium and terbutaline on contractility and K⁺ uptake in isolated human uterine muscle

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Kristjar Skajaa, MD, Maria E. Everts, PhD, Torben Clausen, MD, PhD, and Axel Forman, MD, PhD Aarhus. Denmark

Therapeutic concentrations of Mg⁺⁺ and terbutaline effectively inhibited contractions in isolated human pregnant myometrium by mechanisms independent of the Na⁺-K⁺ pump.

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Beerse, Belgium

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Role of endogenous atrial natriuretic factor in the regulation of fetal cardiovascular and renal function

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Cecilia Y. Cheung, PhD La Jolla, California

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A rabbit model for bacterially induced preterm pregnancy loss: Intervention studies with ampicillin-sulbactam

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Robert S. McDuffie, Jr., MD, Sandra J. Blanton, MS, Robert H. Shikes, MD, and Ronald S. Gibbs, MD Denver, Colorado

When pregnant rabbits at 70% gestation were inoculated hysteroscopically with *Escherichia* coli, treatment with ampicillin-sulbactam resulted in significantly less pregnancy loss than no treatment.

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Editors' note

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

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PREMARIN® Brand of conjugated estrogens tablets, USP PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that natural "estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses. 2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female tetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.

There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares urine It contains estrone, equilin, and 17a-dihydroequilin, together with smaller amounts of 17a-estradies equilenin, and 17a-dihydroequilenin as salts of their sulfate seters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated

estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism,

castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and

PREMARIN (conjugated estrogens) vaginar cream is more accounted by valvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions:

1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogenent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thrombophlebitis of thrombophlebitis and thrombophlebitis and thrombophlebitis and thrombophlebitis and thrombophlebitis disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below).

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophtebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose. Blood pressure should be monitored with estrogen use, especially it high doses are used. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion for progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects on examination and a complete medical and family history should be taken prior to the initiation of any

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should

include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dystunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine lelomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (rotal contraceptives):

combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-

1. Increased prointmonia and actors vii, viii, X, and X, decreased antitriumbun 3, increased interprine induced platelet aggregability.
 2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T₄ levels determined by column or by radioimmunoassay. Free T₃ resin uptake is decreased, reflecting the elevated TBG; free T₄ concentration is unaltered.
 3. Impaired glucose tolerance.
 4. Reduced response to metyrapone test.
 5. Reduced serum folder concentration.

5. Reduced serum totate concentration. MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See CONTRAINDICATIONS and Paved Warning.

PRESHANCY CATEGORY X: Estrogens should not be used during pregnancy. See CONTRAINDICATIONS and Boxed Warning MUTSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine libromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erytherma multiforme, erytherma nodosum, hemorrhagic eruption, loss of scalp hair, histuitism; stepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. Given cyclically for short-term use only. For treatment of moderate-to-severe vasomotor symptoms, alrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be made at three- to six-month intervals.

2. Given cyclically: Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis.—0.625 mg daily. Administration should be exclic feet, three weeks on and one week off).

Osteoporosis.—0.625 mg daily. Administration should be cyclic (eg. three weeks on and one week off).

PREMARIN* Brand of conjugated estrogens Vaginal Cream
Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as

In the lowest use with control symptoms anothe be made at three- to six-month intervals.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an infact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

Revised August 21, 1989

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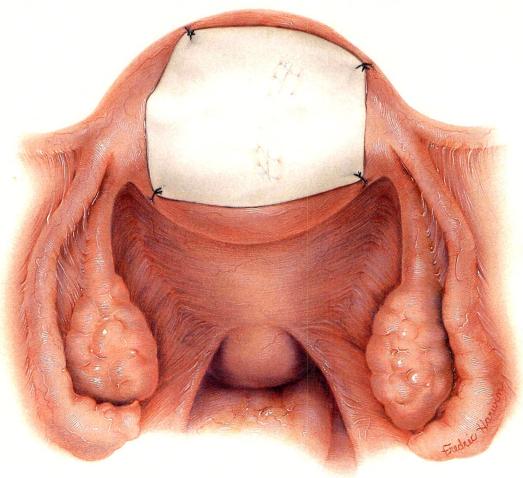
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Two types of letters will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication.

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These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

- —The original and two copies of the manuscript are submitted.
- —The completed checklist is enclosed. The assignment of copyright signed by all authors accompanies the manuscript.
- —Human experimentation has been approved by the local institution as stated in the *Material and Methods* section.
- —Guidelines for the care and use of nonhuman animals or other species approved by the institution have been followed as indicated in the Material and Methods section. The species is named in the title, abstract, key words, and Material and Methods section.
- —The covering letter with essential information is with the manuscript.
- —All elements of the manuscript are typed in English double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
- —All pages are numbered in the following order: title page, condensation, structured or standard abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
- —Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
- —Signed, written permission for the use of quotations of personal communications and unpublished data has been obtained from the person(s) being quoted and is enclosed.

Authorship

—In the covering letter that accompanies the submitted manuscript I/we have confirmed that all authors fulfilled both conditions required for authorship.

Conflict of interest

—In the covering letter that accompanies the submitted manuscript the commercial association of the author or of any coauthors that might pose a conflict of interest is described.

Previous publications

- Enclosed with the submitted manuscript are two reprints each of articles the author and/or some of the coauthors have previously published, have submitted for possible publication, or have in manuscript form dealing with same patients, same animals, same laboratory experiment, or same data, in part or in full, as those reported in the submitted manuscript. Further explanation is provided in the covering letter that accompanies the submitted manuscript.
- -Similarities, differences and explanation are provided in

the covering letter that accompanies the submitted manuscript.

Reviewers

—Names and addresses of three suggested reviewers are enclosed.

Title page

- -These elements are given in the following sequence, and are typed double-spaced.
- -Title.
- —Author(s) name(s) and highest academic degree(s) are shown.
- —City(ies), state(s), and country other than United States in which the study was conducted are given.
- —The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the institutional affiliation(s) of the author(s) at the time of the study is indicated.
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- —At the bottom of the page, a running head not exceeding 52 characters (including word spaces) has been typed.

Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract and key words or short phrases

- -The abstract (structured or standard format) is typed double-spaced with required margins on page 3 headed by the title and author(s) name(s). Beneath the abstract 3 to 5 key words or short phrases are typed.
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- —A standard abstract is submitted as required for Clinical Opinion and Current Development articles with 50 to 150 words and for case reports and brief communications articles with a maximum of 50 words, be they independent or society articles.

References

- -Are typed double-spaced.
- —Are numbered consecutively in the order they are cited in the text.
- —The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors have been followed.
- —Personal communications and unpublished observations are not used as numbered references but are mentioned in the text with the written approval of the person being quoted. The signed approval is enclosed.

Figures

- —Each is numbered with an Arabic numeral and cited in numeric sequence in the text.
- —Three sets of unmounted glossy prints for black-andwhite figures are submitted, properly numbered, labeled, and top indicated on the back of each print.

- —Three sets of original computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back and *top indicated* are submitted. None are dot matrix or photographic prints.
- —Original transparencies of *color photographs* appropriately numbered and identified and *top indicated* are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

Figure legends

- -Are provided for each figure.
- —Are numbered and typed together in numeric order on one sheet of paper (more if necessary). The page is numbered in sequence after the References page(s).

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AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

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Fellowships

The American Board of Obstetrics and Gynecology, Inc.: Approved fellowship training programs in obstetrics and gynecology subspecialties: Gynecologic Oncology; Reproductive Endocrinology; Maternal-Fetal Medicine

CATEGORY State: City Institution	Number of approved positions	Director of program
GYNECOLOGIC ONCOLOGY		
Alabama: Birmingham		
Univ Ala	1 per year (total 2)	Edward E. Partridge
California: Los Angeles		
LAC/USC Med Ctr	1 per year (total 2)	C. Paul Morrow
UCLA	1 per year, alt 2 per year (total 3)	Jonathan S. Berek
California: Orange		
Univ Calif Irvine	1 per year, alt 2 per year, alt 1 per year (total 4, alt 5) (3 year program)	Michael Berman
Connecticut: New Haven		
Yale Univ	1 per year (total 2)	Peter E. Schwartz
DC: Washington		
Georgetown Univ	1 per year (total 2)	Gregorio Delgado
Walter Reed Army Med Ctr	1 per year (total 2)	Robert C. Park
Florida: Miami		
Univ Miami	l per year, alt 2 per year (total 3)	Hervy E. Averette
Florida: Tampa	•	
Univ So Fla	1 per year (total 2)	Denis Cavanagh
Iowa: Iowa City		
Univ Iowa Hosp & Clinics	1 per year (total 2)	Barrie Anderson
Kentucky: Lexington		
Univ Ky	1 per year (total 2)	John R. van Nagell, Jr.
Maryland: Baltimore		
Johns Hopkins Univ	1 per year (total 2)	John L. Currie
Massachusetts: Boston		
Brigham & Women's Hosp	1 per year (total 2)	Ross Berkowitz
Mass Gen Hosp	1 per year (total 2)	Arlan F. Fuller
Michigan: Ann Arbor		
Univ Mich	1 per year (total 2)	James A. Roberts
Minnesota: Minneapolis		
Univ Minn	l per year, alt 2 per year (total 3)	Linda Carson
Minnesota: Rochester		
The Mayo Clinic	l per year (total 3) (3 year program)	Karl C. Podratz
Missouri: St Louis		
Washington Univ	1 per year (total 2)	Ming-Shian Kao
New York: Bronx		
Albert Einstein Coll Med	l per year (total 3) (3 year program)	Carolyn D. Runowicz
New York: Brooklyn		* 1
SUNY Health Sci Ctr/Brooklyn	1 per year (total 2)	John G. Boyce
New York: Buffalo		N. C
Roswell Park Cancer Inst	1 per year (total 2)	M. Steven Piver
New York: New York	1.0	C. L. C.P.U
Memorial Sloan-Kettering	1 per year, alt 2 per year, alt 1 per year (total 4, alt 5) (3 year program)	Stephen C. Rubin
Mt Sinai Med Ctr	1 per year (total 2)	Carmel J. Cohen
New York: Rochester	- •	
Univ Rochester	1 per year (total 2)	Brent DuBeshter

CATEGORY		
State: City	* * * * * * * * * * * * * * * * * * * *	
Institution	Number of approved positions	Director of program
GYNECOLOGIC ONCOLOGY—Con	t'd	
North Carolina: Chapel Hill	1	
Univ NC	I per year (total 2)	Wesley C. Fowler, Jr.
North Carolina: Durham		,
Duke Univ	1 per year (total 2)	Daniel L. Clarke-Pearson
Pennsylvania: Hershey		
Milton S. Hershey Med Ctr	1 per year (total 2)	Rodrigue Mortel
Pennsylvania: Philadelphia	1 man upon (total 9)	Taba I Milanta
Hosp Univ Pa Fexas: Dallas	l per year (total 2)	John J. Mikuta
Univ Tex/Southwestern	l per year (total 3)	David S. Miller
omv rear bounivestern	(3 year program)	David S. Willer
Texas: Houston	,	
M. D. Anderson Cancer Center	3 per year (total 6)	J. Taylor Wharton
REPRODUCTIVE ENDOCRINOLOG	SY .	
California, La Jalla	•	• • • • • • • • • •
California: La Jolla Univ Calif San Diego Med School	2 per year (total 4)	Samuel S.C. Yen
California: Los Angeles	2 per year (total 4)	Samuel S.G. Ten
LAC/USC Med Ctr	3 per year (total 6)	Rogerio A. Lobo
UCLA/Cedars-Sinai Med Ctrs	2 per year (total 4)	Howard L. Judd
California: Orange		.
Univ Calif Irvine Med Ctr	1 per year (total 2)	Ricardo H. Asch
California: San Francisco	•	
Univ Calif San Francisco	3 per year (total 6)	Robert B. Jaffe
California: Torrance	1	
Harbor-UCLA Med Ctr	l per year, total 2	Oscar A. Kletzky
Connecticut: Farmington Univ Conn	1 per year (total 2)	Anthony A. Luciano
Connecticut: New Haven	i per year (total 2)	Anthony A. Luciano
Yale Univ	3 per year, alt 4 per year (total 7)	Michael P. Diamond
DC: Washington	r r r r r r r r r r r r r r r r r r r	
George Washington Univ	1 per year (total 2)	Robert J. Stillman
Georgetown Univ School Med	1 per year (total 2)	James A. Simon
Walter Reed Army Med Ctr	3 per year (total 6)	Thomas A. Klein
Florida: Tampa		
Univ So Fla	1 per year, total 2	George B. Maroulis
Georgia: Augusta	9 non your (total 4)	" PI C M-D I
Med Coll Ga Illinois: Chicago	2 per year (total 4)	Paul G. McDonough
Humana Hosp/Michael Reese	2 per year (total 4)	Edward L. Marut
Northwestern Univ	1 per year (total 2)	Anne Colston Wentz
Rush Med Coll	2 per year (total 4)	Ewa Radwanska
Univ Chicago	1 alt years (total 1, alt 2) (3 year	Randall B. Barnes
. •	program)	
Iowa: Iowa City		
Univ Iowa	I per year (total 2)	F. K. Chapler
Kentucky: Lexington		Company and
Univ Ky	1 per year (total 2)	Ken Muse
Kentucky: Louisville Univ Louisville	I per year (total 9)	Manufa A Manua
Maryland: Baltimore	1 per year (total 2)	Marvin A. Yussman
Johns Hopkins Univ School Med	3 per year (total 6)	Howard A. Zacur
Univ Md	1 per year (total 2)	Eli Y. Adashi
	- For Jour (cours m)	AM I. INGOIN

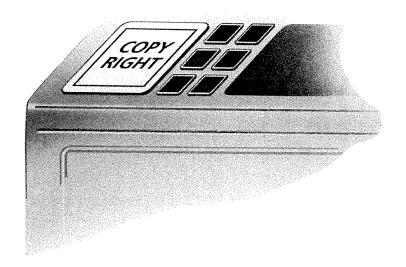
CATEGORY		
State: City Institution	Number of approved positions	Director of program
REPRODUCTIVE ENDOCRINOLO	GY—Cont'd	
Massachusetts: Boston		
Brigham & Women's Hosp	3 per year (total 6)	Kenneth J. Ryan
Michigan: Detroit Wayne State Univ	1 per year, alt 2 per year (total 3)	Kamran S. Moghissi
Minnesota: Rochester The Mayo Clinic	1 per year (total 2)	Steven J. Ory
Missouri: St Louis Washington Univ	1 per year (total 2)	Ronald C. Strickler
New Jersey: New Brunswick		
, UMDNJ/Robt Wood Johnson New Jersey: Newark	1 alt years (total 1)	Ekkehard Kemmann
UMDNJ/New Jersey Med School New York: New York	2 per year (total 4)	Nanette Santoro
Coll Phys Surg/Columbia Univ	1 per year (total 2)	Raphael Jewelewicz
Mt Sinai Med Ctr	1 per year (total 2)	Lawrence Grunfeld
NY Hosp/Cornell Univ	1 per year, alt 2 per year (total 3)	Zev Rosenwaks
North Carolina: Chapel Hill Univ NC	1 per year (total 2)	Mary G. Hammond
North Carolina: Durham Duke Univ	1 per year, alt 2 per year (total 3)	Arthur F. Haney
Ohio: Cincinnati	r per year, are 2 per year (team ey	
Univ Cincinnati Ohio: Columbus	1 per year (total 2)	James H. Liu
Ohio State Univ	1 per year, alt 2 per year (total 3)	Chad I. Friedman
Oregon: Portland		•
Oregon Health Sci Univ Pennsylvania: Philadelphia	1 per year (total 2)	Kenneth A. Burry
Hosp Univ Pa	3 per year (total 6)	Luigi Mastroianni, Jr.
Tennessee: Memphis Univ Tenn	1 per year (total 2)	John E. Buster
Texas: Dallas	9 non woon alt 3 non woon (total 5)	Bruce R. Carr
Univ Tex/Southwestern Texas: Houston	2 per year, alt 3 per year (total 5)	
Baylor Coll Med Texas: San Antonio	2 per year (total 4)	C. James Chuong
Univ Tex/San Antonio Vermont: Burlington	1 per year (total 2)	Robert S. Schenken
Univ Vt Virginia: Charlottesville	1 per year (total 3) (3 year program)	John R. Brumsted
Univ Va Virginia: Norfolk	1 alt years (total 1)	James D. Kitchin III
Eastern Va Med School	2 per year (total 4)	Charles C. Coddington
Wisconsin: Madison Univ Wis	1 per year (total 2)	Sander S. Shapiro
Canada: Montreal (Que) McGill Univ	1 per year (total 2)	A. Brian Little
MATERNAL-FETAL MEDICINE	<i>r</i>	
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Alabama: Birmingham Univ Ala	1 per year, alt 2 per year (total 3)	John C. Hauth
Arizona: Tucson Univ Ariz	1 per year (total 2)	Kathryn L. Reed

CATEGORY		
State: City Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE—C	ont'd	
•	· .	
Arkansas: Little Rock Univ Ark	I per year (total 2)	J. Gerald Quirk, Jr.
California: La Jolla	i per year (total z)	J. Gerald Quirk, Jr.
Univ Calif San Diego Med Ctr	1 per year, alt 2 per year (total 3)	Thomas R. Moore
California: Loma Linda		
Loma Linda Univ	1 per year (total 2)	Barry Block
California: Los Angeles	- , ·	,
LAC/USC Med Ctr	4 per year (total 8)	Richard H. Paul
UCLA	l per year (total 2)	Brian J. Koos
California: Orange		
Univ Calif Irvine/Long Beach Hosp	3 per year (total 6)	Thomas J. Garite
California: San Francisco		
Univ Calif San Francisco	4 per year (total 8)	Julian T. Parer
California: Torrance		
Harbor-UCLA/Cedars-Sinai	3 per year (total 6)	Michael Ross
Colorado: Denver	0 (4.14)	,
Univ Colo	2 per year (total 4)	Roger L. Lenke
Connecticut: Farmington	2 per year (total 4)	A male amore N.C. William II amore
Univ Conn Connecticut: New Haven	2 per year (total 4)	Anthony M. Vintzileos
Yale Univ	l per year (total 2)	John C. Hobbins
DC: Washington	i per year (total 2)	John C. Hobbins
George Washington Univ	1 per year (total 2)	John H. Grossman III
Georgetown Univ	l per year (total 2)	John T. Queenan
Florida: Gainesville	i por jeur (tour 1)	John 1. Queenun
Univ Fla	1 per year (total 2)	Amelia C. Cruz
Florida: Miami	,	
Univ Miami	2 per year (total 4)	Mary Jo O'Sullivan
Florida: Tampa		, , , , , , , , , , , , , , , , , , , ,
Univ So Fla Coll Med	3 per year (total 6)	William F. O'Brien
Georgia: Atlanta		
Emory Univ School Med	1 per year (total 2)	John F. Huddleston
Georgia: Augusta	•	· ·
Med Coll Ga	1 per year (total 2)	Lawrence D. DeVoe
Illinois: Chicago		
Northwestern Univ Med Ctr	2 per year, alt 3 per year (total 5)	Michael L. Socol
Rush Presbyterian/St Lukes	1 per year (total 2)	Howard T. Strassner, Jr.
Univ Chicago	1 per year (total 2)	Mark Phillippe
Univ Ill	2 per year (total 4)	Andre Bieniarz
Illinois: Springfield		
Southern Ill Univ	1 per year (total 2)	Robert C. Kaufmann
Indiana: Indianapolis	1 (10)	
Indiana Univ School Med	1 per year (total 2)	Alan M. Golichowski
Iowa: Iowa City	1 (1 9) (9	O in tu'
Univ Iowa Kentucky: Louisville	1 per year (total 3) (3 year program)	Carl P. Weiner
	9 per year (total 4)	Iosanh A Sminner
Univ Louisville School Med Louisiana: New Orleans	2 per year (total 4)	Joseph A. Spinnato
La State Univ	I per year (total 9)	Hamiou A. Cohom
Maryland: Baltimore	1 per year (total 2)	Harvey A. Gabert
Johns Hopkins Univ	2 per year (total 4)	Timothy D D Johann
Univ Md	1 per year (total 4) 1 per year, alt 2 per year (total 3)	Timothy R. B. Johnson
CTILL TITE	i per year, an i per year (total 3)	David A. Nagey

CATEGORY		
State: City		
Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE—C	ont'd	•
Massachusetts: Boston		
Brigham & Women's Hosp	2 per year (total 4)	Fredric D. Frigoletto, Jr.
Tufts Univ/St Margaret's	2 per year (total 4)	Mary E. D'Alton
Michigan: Ann Arbor		
Univ Mich Med Ctr	1 per year (total 3) (3 year program)	Robert H. Hayashi
Michigan: Detroit		
Wayne State Univ	2 per year (total 4)	Sidney F. Bottoms
Mississippi: Jackson		
Univ Miss	2 per year (total 4)	James N. Martin, Jr.
Missouri: St Louis		
Washington Univ Med Ctr	3 per year (total 6)	D. Michael Nelson
New Jersey: New Brunswick		
UMDNJ/Robt Wood Johnson	1 per year (total 2)	John T. Harrigan
New Jersey: Newark		
UMDNJ/NJ Med School	1 per year (total 2)	Joseph J. Apuzzio
New Mexico: Albuquerque		
Univ NM	2 per year (total 4)	Luis B. Curet
New York: Bronx		
Albert Einstein Coll Med	2 per year, alt 3 per year (total 5)	Irwin R. Merkatz
New York: Brooklyn	•	
SUNY Health Sci Ctr/Brooklyn	1 per year (total 2)	Howard L. Minkoff
New York: Buffalo	•	
SUNY Health Sci Ctr/Children's	1 per year (total 2)	William P. Dillon
Hosp		
New York: New York		
Columbia Univ	2 per year (total 4)	Harold E. Fox
Mt Sinai School Med	1 per year, alt 2 per year (total 3)	Richard L. Berkowitz
NY Hosp/Cornell Univ	l per year (total 2)	Frank Chervenak
NY Univ	l per year (total 2)	Bruce K. Young
New York: Rochester		
Univ Rochester	l per year, alt 2 per year (total 3)	James R. Woods, Jr.
New York: Stony Brook		
SUNY Health Sci Ctr/Stony Brook	1 per year (total 2)	David A. Baker
New York: Valhalla		
NY Med Ctr/Westchester Cnty	1 per year (total 2)	Nergesh Tejani
North Carolina: Chapel Hill		
Univ NC	1 per year, alt 2 per year (total 3)	Robert C. Cefalo
North Carolina: Durham		
Duke Univ	1 per year, alt 2 per year (total 3)	Allen P. Killam
North Carolina: Winston-Salem		
Bowman Gray School Med	1 per year (total 2)	J.C. Veille
Ohio: Cincinnati		
Univ Cincinnati	2 per year (total 4)	Tariq A. Siddiqi
Ohio: Cleveland		
MetroHealth Med Ctr	2 per year (total 4)	LeRoy J. Dierker, Jr.
Univ/MacDonald Womens Hosp	1 per year (total 2)	Method Duchon
Ohio: Columbus		
Ohio State Univ	1 per year (total 2)	Jay D. Iams
Oklahoma: Oklahoma City		
Univ Ok Coll Med	l per year (total 2)	Gary R. Thurnau
Oregon: Portland	•	
Ore Health Sci Univ	1 per year (total 2)	Richard I. Lowensohn
	* · ·	

CATEGORY		
State: City		12
Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE	—Cont'd	
Pennsylvania: Philadelphia		
Hosp Univ Pa	2 per year (total 4)	Arnold W. Cohen
Pa Hosp	2 per year (total 4)	Stuart Weiner
Thomas Jefferson Univ	2 per year (total 4)	Ronald J. Wapner
Pennsylvania: Pittsburgh		*
Univ Pittsburgh	2 per year (total 4)	Daniel I. Edelstone
Rhode Island: Providence		
Brown Univ	1 per year (total 2)	Marshall W. Carpenter
South Carolina: Charleston		·
Med Univ SC	1 per year (total 2)	Roger B. Newman
Tennessee: Memphis		J
Univ Tenn	2 per year (total 4)	Baha M. Sibai
Tennessee: Nashville		•
Vanderbilt Univ	1 per year (total 2)	Frank H. Boehm
Texas: Dallas		
Univ Tex/Southwestern	3 per year, alt 4 per year (total 7)	Larry C. Gilstrap III
Texas: Houston	• •	· ·
Baylor Coll Med	3 per year (total 6)	Kenneth J. Moise, Jr.
Univ Tex/Houston	3 per year (total 6)	Valerie M. Parisi
Texas: San Antonio		
Univ Tex/San Antonio	2 per year (total 4)	Edward R. Newton
Utah: Salt Lake City		
Univ Utah	1 per year (total 3) (3 year program)	D. Ware Branch
Vermont: Burlington		
Univ Vt	1 per year (total 3) (3 year program)	Eleanor L. Capeless
Virginia: Norfolk		•
Eastern Va Med School	I per year (total 2)	Donald L. Levy
Washington: Seattle	* ,	
Univ Wash	1 per year (total 2)	Thomas J. Benedetti
Washington: Tacoma		
Madigan Army Med Ctr	1 per year (total 2)	Jerome N. Kopelman
Wisconsin: Madison	(L) /	3
Univ Wis	1 per year (total 2)	Chester B. Martin, Jr.

Fellowships are listed alphabetically by state.



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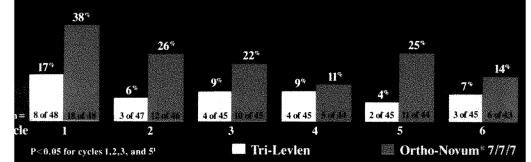
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inclinations and Usage
Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

ntraindications

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Oral confraceptives should not be used in women with any of the following conditions: thrombophiebits or thromboembolic disorders, a past history of deep-ven thrombophiebits or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected corcinoma of the breast, carcinoma of the endometrium or other known or suspected estragendependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic joundice of pregnancy or joundice with prior use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

who use oral confraceptives should be strongly acrived not to smoke.

The use of oral confraceptives as associated with increased risks of several senious conditions including myocardial infarction, thromboembolism, stroke, hepotic neoplasia, galiblodder disease, and hypertension, although the risk of senious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and morbidity increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemics, obesity and diabetes Practitioners prescribing oral confraceptives should be familiar with the following information relating to these risk indistriction contained in this package insert is principally based on studies corried out in patients who used oral confraceptives with higher formulations of estragens and progestagens than those in common use today. The effect of long-fermuse of roal confraceptives with lower formulations of both estragens and progestagens remains to be determined. Throughout this labeling, epidemiological studies provide are of two hypes: retrospective or case control studies and prospective or cohort studies accurate and the provide information on the actual clinical occurrence of a disease of measure of the relative risk does not provide information on the actual clinical occurrence of a disease control studies provide in a control of the provide information. For further information, the reader is referred to a text on epidemiological methods.

1. **Innomboembolic Disorders and Other Vascular Problems**

in the population. For further information, the reader is referred to a fext on epidemiological methods.

1. Thromboembolic Disorders and Other Vascular Problems
a. Myccardial Infanction. An increased risk of myccardial infanction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hyper-cholesterolemial, morbid obesity, and diabetes. The relative risk of heart dirack for current oral-contraceptive use has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infanctions in women in their mid-finities or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives or oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyper-lipidemias, age and obesity. In particular, some progestagens are known to decrease HDL cholesterol and cause glucose infolerance, while estingens may create a state of hyperimalunism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heard disease oral contraceptives must be used with coution in women with cordiovascular disease isk factors.

Introducerous and the subsequent of the propertion of an attendance of several calendary and an advanced and thromboembolic and thromboembolic and thromboembolic and thromboembolic and thromboembolic and thromboembolic and thromboembolic and thromboembolic and thromboembolic and

risk of heard disease. Oral contraceptives must be used with coution in women with caralovascular disease risk factors.
Intromberabolism. An increased risk of thromboembolic and thrombotic disease associated with the so of oral confraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thiombosis. 4 to 11 for deep vein intrombosis or pulmonary embolism, and 1 5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4 5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of enous histority or women who have predisposing conditions is tivice that of women without such medical conditions. If feasible, and confraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, and contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

should be started no earlier than four to six weeks after delivery in women who elect not to breast-teed, or a midtrimester pregnancy termination.

Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is gredest among older (> 35 years), hypertensive women who also smoke Hypertension was found to be a risk factor for both users and nonusers, for both hypes of strokes will be smoking interacted to increase the risk for hemorrhagic strokes in a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to a for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used and contraceptives, 2.6 for smokers who did not use oral contraceptives, 1.6 for smokers who add not use oral contraceptives, 1.6 for smokers who used oral confraceptives, 2.6 for smokers who severe hypertension. The attributable risk is also greater in older women.

I observe related risk of vascular disease form and contraceptives. A positive association has been observed between the amount of estrogen and progestogen in and contraceptives and the risk of vascular disease. A decline in serum high-density ipoproteins has been associated with an increased incidence of ischemic heart disease Because estrogens and progestogen of the nature and obsolute amount of progestogen and employed progestogen and progestogen and the nature and obsolute amount of progestogen and several progestory of the amount of progestogen and the nature and obsolute amount of progestogen services and progestogen and progestogen in the contraceptive depends on a both of the analysis of the considered in the choice of an oral contraceptive depends and progestogen and progestogen is in keeping with good

e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for e reassence or insk of vascular disease intere are two studies which have shown persistence of risk of vascular disease for ever users of oral contraceptives in a study in the United States, the risk of developing myocardial infarction discontinuing oral contraceptives persists for all east 9 years for women 40-49 years who had used and contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for all least 6 years after discontinuation of aral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estracens.

ot estrogens. Outstanders were perionted with concurrence formations committed to the controlled of the consocial with different methods of controception at different ages. These estimates include the combined risk of death associated with different methods of controception at different ages. These estimates include the combined risk of death associated with controceptive methods plus the risk attributable to pregnancy in the exent or method foliure Each method of controception has its specific benefits and risks. The study concluded that with the exception of in-controceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of brith control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age to crai-controceptive users is based on dara gathered in the 1970's – but not reported until 1983. However, current clinical practice involves the use of lower estrogen does formulations combined with careful restriction of oral-contraceptive use to women who do not have the various ists factors isted in this labeling. Because of these changes in practice and, also, because of some imitted new data which suggest that the risk of contiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Moternal Health Drugs Advisory Committee vas asked to review tops. In the processing of the controlled with oral-contraceptive use for age effect and of an health pronouncy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have excess to effective and acceptable means of contraception. Therefore, the controlled between a controlled to the lowest possible dose formulation that is effective.

is effective

3. Carcinoma of the Reproductive Organs. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarion and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast concer, regardless of the age and partly of list use or with most of the marketled brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for of least a decade following large-term use. A few situaties have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonursers and differences in age at start of use, has been destained. Some studies suggest that and oral-contraceptive use has been associated with an increase in the risk of cervical intrapsified neoplassia in some populations of women. However, there continues to be controversy about the extent to which such indings may be due to differences in sexual behavior and other factors in spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. Heactic Neopolassia Benian headtic adenomous oral essociated with an increase in the sufficiency to the incidence of benian.

4 Hepatic Neoplasia Bengin hepatic adenorms are associated with oral-contraceptive use, eithough the incidence of bengin tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/ 100.000 for users, a risk that increases after four or more years of use Rupture of rare, bengin, hepatic adornous may cause death through intro-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatic-cellular.

carcinoma in long-term (> 8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users. 5 *Coular Lesions*. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision, onset of prophosis or dipipion, oppliedema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

token immediately.
6. Oral-Controceptive Use Before or During Early Pregnancy Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral confraceptives prior to pregnancy Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral confraceptives to induce withdrawal bleeding should not be used as lest for pregnancy. Oral confraceptives should not be used so lest for pregnancy. Oral confraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed have consecutive periods, pregnancy should be ruled out before continuing oral-confraceptive use if the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-confraceptive use should be discontinued if pregnancy is confirmed.

7. Galibiadder Disease Earlier studies have reported an increased lifetime relative risk of authoridate supregrup users of oral.

To allibidate Disease Earlier studies have reported an increased lifetime relative risk of galibidater surgery in users of oral controceptives and estragens. More recent studies, however, have shown that the relative risk of developing galibidater disease among prol-contraceptive users may be minimal. The recent findings of manimal isk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estragens and progestagens.

tormulations containing lower hormanal doses of estrogens and progestagens.

8. Carbohydrate and Lipid Medabolic Effects. Oral contraceptives have been shown to cause grucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hypernsulinism, white lower doses of estrogen cause less glucose intolerance Progestagens increase insulin secretion and create insulin resistance, his effect varing with different progestational agents. However, in the nondiabetic women, rario contraceptives appear to have no effect on tasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking and contraceptives. A small proportion of women with have persistent hyperfrighycendemia while on the pill. As discussed earlier (see "Womings" to and bt.), changes in serum frighycendes and lipoprotein levels have been reported in oral-contraceptive users.

9. Elevated Blood Pressure. An increase in blood pressure has been reported in women taking and contraceptives and this increase is more likely in older and-contraceptive users and with continued use. Data from the Roya: College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestagens. Women with a history of hypertension or hypertension either diseases, or real diseases should be encouraged to use another method of contraception. If women with hypertension elect to use and contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, and contraceptives, and there is no difference in the occurrence of hypertension on manna elevation of blood pressure occurs, and contraceptives, and there is no difference in the occurrence of hypertension on generand near-users.

or imperioration unity generation for use the control of the control of the data the with a new pattern that is recurrent, persistent or severe requires discontinuation of oral controceptives and evaluation of the cause.

Tell of severe requires aiscommunion or ora-commonships and evaluation or included in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestagen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of break-through bleeding, as in the case of any abnormal vaginal bleeding; if pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or alignmenorrhea, especially when such a condition was preexistent.

tormulation may solve the procern. In the event of amenormed, pregnancy snows are ruled out some women may encounter post-full amenormed or oligomenormed, especially when such a condition was preexistent.

Procurtions

1. PHYSIGAL EXAMINATION AND FOLLOW UP A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests in case of undiagnosed, persistent or recurrent abnormal vaginal bleeding opportune diagnoses and interest of the process of the control of hyperpropriate diagnoses and interest of the process of

Information for the Patient See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reachons has been associated with the use of oral contraceptives (see "Warnings" section) inhornbopinethetis, arterial thromboers been pulmonary embolism, impropriate inhornbost, by the propriet is a pulmonary embolism, impropriate inhornbost, hyperfension, galibilitader disease, heptic adenomos or bening liver trumors.

There is evidence at an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed inesenteric thrombosis; retinal thrombosis.

The following adverse reactions have been reported in options receiving and contraceptives and are believed to be drug related nausea, worlding, gastrointestinal symptoms (such as abdominal cramps and blooting), breakthrough bleeding, spotting, change in menstrual flow, amenorthea, temporary infertility after discontinuation of treatment, edema, melasma which may persist, breast changes tenderness, enlargement, secretion, change in weight (increase or decrease), change in cervical erosion and secretion, diminution in lactation when given immediately postportum, cholestatic journaice, migratine, rosh (altergic), mental depression; reduced tolerance to carbothydrates, vaginal candidiasis; change in corneal curvature (steepening), intolerance to control tenses. to contact lenses

to confact lenses.

The following adverse reachins have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted, congenital anomalies, premenstrual syndrome, cataracts, apitic neuritis, changes in appetite, cystitis-like syndrome, headache, nenousness, dizziness, hissultism, loss of scalip hair, erythema multiforme, cerebral vascular disease with mitral valve prolopse, fupus-like syndromes, erythema noolosum, hemorrangic eruption, organitis, porphyring, impaired renal function, hemolytic uremic syndrome, Budd Chiari syndrome, acne, changes in libido, calitis, sickle-cell disease

Overdosage
Sentous ill effects have not been reported following ocule ingestion of large doses of oral contraceptives by young children
Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits
The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of meeting 0.035 mg of ethinyl estradiol or 0.05 studies which largely talkzed ordi-confraceptive formulations containing doses exceeding 0 035 mg of ethinyl estradiol or 0 05 mg of mestranol.
Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorthea.
Effects related to inhibition of avulation, decreased incidence of functional avarian cysts, decreased incidence of ectopic pregnancies.

narrows Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of avanan cancer Dosage and Administration

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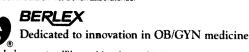
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Reference: 1. Data on file, Berlex Laboratories.



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CLINICAL SECTION

Clinical Opinion

An epidemic of antiabortion violence in the United States

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Los Angeles, California, New York, New York, and Washington, D.C.

From 1977 to 1988, an epidemic of antiabortion violence took place in the United States, involving 110 cases of arson, firebombing, or bombing. The epidemic peaked in 1984, when there were 29 attacks. Nearly all sites (98%) were clinics that provided abortions. Facilities in 28 states and the District of Columbia were involved. The national rate of violence was 3.7 per 100 abortion providers and 7.2 per 100 nonhospital abortion providers. The national ratio of violence per 100,000 abortions performed was 0.6. Arson was both the most frequent (39% of all cases) and the most damaging (mean cost \$141,000) type of violence. The epidemic appears partially attributable to multiple point-source outbreaks of violence caused by small numbers of individuals or groups. Thirty-three persons have been convicted to date. Vigorous prosecution of perpetrators and the reemergence of clinics after damage probably helped to curb the epidemic. (AM J OBSTET GYNECOL 1991;165:1263-8.)

Key words: Abortion; abortion, induced; abortion, therapeutic; abortion providers; violence

Harassment and intimidation of providers of abortion services have become widespread in the United States. For example, 47% of abortion providers experienced at least one type of antiabortion harassment in 1985. These activities have ranged from jamming of telephone lines to picketing the homes of physicians to vandalism.2 In recent years, however, the controversy over abortion has taken a destructive turn, resulting in an epidemic of antiabortion violence across the United States. Since 1977 > 100 attempted or completed arsons and bombings have caused millions of dollars of damage and disrupted the provision of health care to large numbers of women. Because this epidemic of violence directed against health care providers is unique in American medicine and because its medical and social implications are profound, we conducted this study to characterize the epidemic.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine, The Alan Guttmacher Institute, New York, and the National Abortion Federation, Washington, D.C.

Reprint requests: David A. Grimes, MD, Department of Obstetrics and Gynecology, Women's Hospital, 1240 North Mission Road, Los Angeles, CA 90033.

6/1/30674

Material and methods

We defined a case of antiabortion violence as an attempted or completed act of arson, bombing, or firebombing directed against an abortion provider or an organization supportive of abortion rights. Cases were identified through the ongoing surveillance of the National Abortion Federation, with the assistance of the United States Bureau of Alcohol, Tobacco and Firearms. Through interviews with the victims and collaboration with law enforcement officials, we determined the date of the occurrence, the city and state, the name and type of facility or organization, the type of violence committed, the estimated damages, and whether a perpetrator was convicted. This report covers episodes of antiabortion violence from Jan. 1, 1977, to Dec. 31, 1988.

We characterized the epidemic by time, victim, and place. We also examined the acts of violence by type and destructiveness. To portray the "vectors" of this epidemic, we also reviewed the convictions of the perpetrators. We calculated the frequency of antiabortion violence by state using three different denominators: the number of abortion providers, the cumulative number of abortions performed in each state from 1977 to 1988, and the estimated number of women aged 15 to

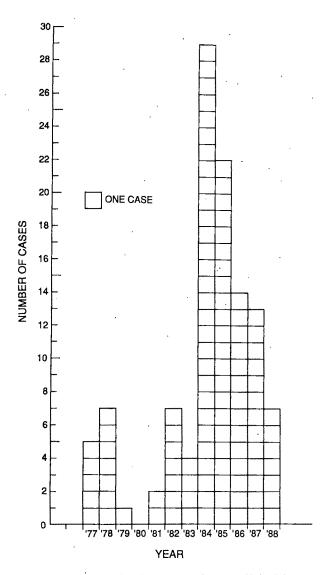


Fig. 1. Cases of antiabortion violence by year, United States, 1977 to 1988.

44 years in each state as of July 1, 1982. Numbers of providers and abortions by state were obtained from surveys by the Alan Guttmacher Institute, ³⁻⁷ with the exceptions of the years 1983 and 1986. Since the institute did not conduct a survey for those years, we used the average number of abortions for 1982 plus 1984 for 1983 and 1985 plus 1987 for 1986. Estimates of the numbers of women of reproductive age (15 to 44) by state for 1982 were obtained from the United States Bureau of the Census. This year was in the middle of the study interval and was selected as a standard for comparison.

To examine the risk of antiabortion violence by type of provider, we calculated violence rates per 100 providers and violence ratios per 100,000 abortions performed. We also sought to identify clusters of cases, which would suggest a common source. For the pur-

poses of our analysis we defined a cluster of cases as two or more cases occurring in the same calendar month in the same state or in a contiguous state. We also examined the correlation between attack rates and percentage change in the number of abortions by state.

Results

From 1977 to 1988, 110 cases of antiabortion violence were identified. The epidemic peaked in 1984, with partial resolution of the epidemic in subsequent years (Fig. 1). Twenty-nine cases occurred in 1984, which was four times the number in any preceding year. One percent of all abortion providers in the United States were targets of attempted or completed arson or bombing in 1984. The ratio of violence in that year was 1.8 episodes per 100,000 abortions.

The targets of antiabortion violence were almost exclusively health care providers. One hundred eight cases (98%) involved providers, whereas the other two victims were organizations related to abortion rights. In July 1984 the office of the National Abortion Federation in Washington, D.C., was damaged extensively by a propane bomb. In November 1984 the Washington, D.C., office of the American Civil Liberties Union was bombed while an employee was present. This attack was classified as abortion related, since the group that bombed the ACLU was convicted of attacking the National Abortion Federation and a number of clinics.

Nonhospital providers (clinics and physicians' offices) were the only providers attacked (Table I). A total of 80 different facilities were attacked, 20% more than once. Nine were attacked twice, three facilities three times, three facilities four times, and one facility five times during the study interval. The number of attacks among these 80 providers was 1.4 ± 0.8 (mean \pm SD).

Antiabortion violence was committed from coast to coast (Fig. 2). Twenty-eight states and the District of Columbia experienced one or more episodes. The largest number of cases occurred in Ohio (n=16), followed by California (n=10), Texas (n=9), and Florida (n=8). However, the highest rate of violence per 100 nonhospital providers occurred in Minnesota, followed in decreasing order by Delaware, Ohio, and North Dakota. Violence ratios (number of cases per 100,000 abortions performed) were highest for Vermont, followed by Oregon, Minnesota, and North Dakota (Table II). The national ratio from 1977 to 1988 was 0.6 cases per 100,000 abortions.

Violence levels by size of state revealed a different pattern. The highest ratios of violence in the 1977 to 1988 period per million women of reproductive age in 1982 were seen in the District of Columbia (17.8), followed by Vermont (15.7), Oregon (11.0), and Minnesota (7.1). The national ratio was 2.0 cases per million women of reproductive age.

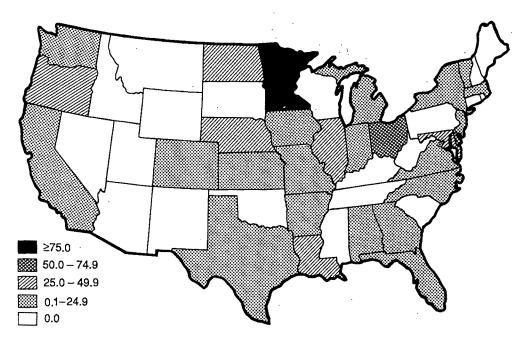


Fig. 2. Rates (number of cases per 100 nonhospital providers) of antiabortion violence against nonhospital providers, by state, United States, 1977 to 1988.

Table I. Rates of antiabortion violence against providers, by type, United States, 1977 to 1988

Type of provider	No. of attacks*	No. of providers†	Rate‡
Hospital Nonhospital	0 108	1405 1503	0.0 7.2
TOTAL	108	2908	3.7

^{*}Excludes two attacks against nonproviders.

Between 1977 and 1988 the number of abortions in the United States increased at an average annual rate of 1.9%. In those areas that had experienced violent attacks against abortion providers, the average annual increase was 2.0%, compared with 1.4% in other states. The correlation between the proportion of abortion providers subject to violent attacks over the 1977 to 1988 time period and the percentage change in the number of abortions between 1977 and 1988 was not significant.

The clustering of episodes both temporally and geographically suggests that the epidemic can be partially explained as multiple point-source outbreaks. Eighteen clusters of cases accounted for 41% of all episodes of antiabortion violence. Forty-five cases were clustered in groups of two to four episodes in the same month in the same locale. Convictions of perpetrators support the multiple point-source outbreak explanation. Several individuals and groups were convicted of multiple

acts of violence. Fifteen individuals or groups (total of 33 persons) have been involved with 42 separate cases (38%). The mean number of cases accounted for by these 15 "vectors" was 2.8, with a range of 1 to 10.

Those convicted were implicated in 8 of the 18 clusters identified epidemiologically, leaving 10 clusters without convictions. One suspect has been indicted on charges related to two arsons but has remained a fugitive because of failure to appear at sentencing on unrelated charges. Two other perpetrators confessed but were not convicted; one was declared incompetent to stand trial and was committed to a state mental institution, and the other was found not guilty by reason of insanity.

The nationwide conviction rate was 39%, although rates varied widely from state to state. All cases resulted in conviction of the perpetrators in the District of Columbia, Maryland, Virginia, Delaware, Indiana, Louisiana, and North Dakota. Some states with large num-

[†]As of 1982.

[‡]Number per 100 providers.

Table II. Ratios of antiabortion violence by state, United States, 1977 to 1988

State	No. of cases of violence*	No. of abortions performed (×1000)	Ratio†	
Vermont	2	42	4.8	
Oregon	7	189	3.7	
Minnesota	7	219	3.2	
North Dakota	· 1	33	3.0	
Ohio	16	705	2.3	
Delaware	I	53	1.9	
Louisiana	4	218	1.8	
Arkansas	1	69	1.4	
Maryland	5	362	1.4	
Nebraska	, 1	77	1.3	
Washington	5	392	1.3	
Florida	8	889	0.9	
Iowa	1	107	0.9	
Illinois	7	825	0.8	
Texas	9	1,171	0.8	
Virginia	3	395	0.8	
Kansas	1	153	0.7	
Alabama	I	220	0.5	
Indiana	1	190	0.5	
Colorado	1	266	0.4	
Georgia	2	447	0.4	
Michigan	2 3	750	0.4	
Missouri	1	232	0.4	
California	10	3,269	0.3	
District of Columbia	1	329	0.3	
New York	6	2,246	0.3	
Massachusetts	1	497	0.2	
North Carolina	1	402	0.2	
New Jersey	1	713	0.1	
United States	108	18,395	0.6	

^{*}Excludes two attacks against nonproviders in the District of Columbia.

bers of cases (Ohio, Oregon, and Texas) failed to convict any perpetrators.

Arson was the most frequent type of violent act. Arson and attempted arson were involved in 39% and 13% of cases, respectively. Bombing (21%) and attempted bombing (15%) were next in frequency. These included one chemical bomb, one propane bomb, one pipe bomb, and one package bomb designed to explode on opening. The package bomb was intercepted at the clinic, and postal officials were notified. Similar packages were found at the local post office addressed to three different clinics. Firebombing (10%) and attempted firebombing (3%) were the least frequent types of violent acts.

Violence involving fire caused more extensive damage than did violence involving explosion. The estimated damages from arson were known for 37 of the 43 cases; the total losses were \$5,215,500 with a mean \pm SD of \$141,000 \pm \$273,700 per case. Damage estimates were available for 7 of the 11 firebombings; the total losses were \$890,000, with a mean \pm SD of \$127,100 \pm \$108,600 per case. Similar data were available for 22 of the 23 bombings; the total losses were

\$1,501,000, with a mean \pm SD of $$68,200 \pm $70,000$ per case. The twofold difference in damages between incendiary and explosive devices is likely an underestimate; several arsons totally destroyed the building, and no replacement value was available. The single most expensive act of violence occurred in Texas in February 1985, when a gasoline-ignited fire destroyed an entire shopping center valued at \$1,500,000 and injured two firefighters.

Comment

One definition of an epidemic is an "unusually frequent occurrence of disease in the light of past experience." By this definition any act of arson or bombing directed against a health care provider or related organization constitutes an epidemic. The epidemic of antiabortion violence in the United States from 1977 to 1988 was a social phenomenon without precedent. To our knowledge this epidemic was the first time in our nation's history that health care providers have been singled out as targets of violence in pursuit of a social agenda. Other controversial areas of medicine, such as life-support systems, organ transplantation,

[†]Per 100,000 abortions performed.

blood banking, and in vitro fertilization, also may be in jeopardy.

Harassment and violence have become part of a strategy of some antiabortion elements; the dramatic increase in violence seen in 1984 has been attributed to their growing frustration by their failure to overturn Roe v Wade and prohibit abortions.2

This frustration was intensified in the summer of 1983 by three factors. The first was a forceful reaffirmation by the United States Supreme Court of a woman's right to abortion and a rejection of restrictions that would limit access to abortion (City of Akron v Akron Center for Reproductive Health, 103 S Ct 2481). The second was the United States Senate's rejection of a constitutional amendment that would allow states to prohibit abortion. The third was the Senate's earlier tabling of the human life statute, which would allow Congress to bypass the constitutional amendment process and to prohibit abortion by another mechanism. These setbacks were difficult for some abortion opponents to accept, given the expectations occasioned by the election of Ronald Reagan to the presidency. Some of the more radical elements gave up hope for judicial or legislative change and instead embarked on a militant course of action.2

Several factors probably helped to curb the epidemic. One was vigorous investigations by local and federal law enforcement agencies, notably, the Bureau of Alcohol, Tobacco and Firearms. The bureau has primary responsibility for federal gun control, explosives control, and antiarson laws. Arrests and convictions may have deterred others. The most severe sentence has been 30 years and the most severe fine \$353,000. Thirty-year sentences were given to two men purporting to be members of a radical antiabortion group known as the "Army of God"; they were convicted of attacks against three abortion clinics and the kidnapping and extortion of a physician. In addition, most facilities that were attacked did not close permanently, so that the disruptions were temporary.

Clinics were the principal targets of antiabortion violence. This finding is consistent with the observation that clinics also are the chief targets of harassment.1 A survey of providers revealed that several factors were significantly related to harassment: type of facility (e.g., clinic vs physician's office), region of the country (higher in the Midwest and South), and proportion of patient visits that involved abortion services. Factors not associated with the likelihood of harassment included number of abortions performed beyond 400 per year, recent change in number of abortions performed, maximum gestational age at which abortions are performed, and cost of the procedure.1 Clinics provide the majority of abortions in the United States and are both visible and vulnerable. Unlike hospitals, which are open for business around the clock, clinics have long periods of time when they are vacant and accessible to attack.

Some states had a disporportionate amount of antiabortion violence. These included both populous states such as Ohio and less populous states such as North Dakota, Delaware, Minnesota, North Dakota, Ohio, and Oregon consistently ranked high in frequency of violence when analyzed according to the number of nonhospital providers, number of abortions performed, and population of women of reproductive age. The District of Columbia had a disproportionately high violence ratio per million women of reproductive age because it has a relatively small population but serves as a referral center for abortion services.

The true cost of this epidemic is hard to estimate. As noted, the direct cost of \$7.6 million is a substantial underestimate because of the exclusion of a number of facilities that were completely destroyed. The related costs of increased expenses for legal and security services, increased fire and casualty insurance, new licensing requirements, and staff recruitment have not been estimated but are large. In addition, the indirect costs of time lost from work during repair and reconstruction are substantial. Patients seeking abortion or other services were forced to postpone care or transfer to another provider. The cost of investigation, prosecution, and incarceration of perpetrators also is large. These costs may translate into higher fees for patients and rising costs of law enforcement.

Even though the costs of this epidemic have been great and some facilities have had to interrupt or discontinue providing abortion services, the violent attacks have not led to fewer women having abortions. There is no statistically significant relationship between the change in the number of abortions between 1977 and 1988 and the levels of violence among nonhospital pro-

This epidemic of antiabortion violence represents a minority of the hostile acts against abortion providers during the study interval. Between 1977 and 1988, the following incidents were reported to the National Abortion Federation: clinic invasion, 222; clinic vandalism, 220; bomb threats, 216; death threats, 65; assault and battery, 46; burglary, 20; and kidnapping, 2. The actual number of hostile acts may be substantially higher, because these figures reflect only voluntary reports.

The majority of United States citizens oppose violence against abortion facilities.1 Clearly, the public suffers from this violence, which is sometimes indiscriminate. For example, a Planned Parenthood Clinic in Georgia was attacked by a firebomb in September 1984; the clinic does not provide abortions but, rather, counseling and contraception that help to avoid the need for abortion.

Abortion will likely remain one of the most contro-

versial social issues of our time. Arson, bombing, and millions of dollars of damage to health care facilities have neither contributed to the debate over abortion nor decreased the numbers of abortions performed. Sex education, personal responsibility, and better contraceptive practices will help to reduce the need for abortions; Molotov cocktails will not. When this is understood, the epidemic of antiabortion violence may finally end.

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Multifetal pregnancy reduction and disposal of untransplanted embryos in contemporary Jewish law and ethics

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Recent responsa (rabbinical rulings) on multifetal pregnancy reduction and disposal of untransplanted embryos indicate that the position of contemporary Jewish law and ethics on these specific issues differs strongly from the *Instruction* of the Roman Catholic Church and is closer to the view adopted by the Ethics Committee of the American Fertility Society. (AM J OBSTET GYNECOL 1991;165:1268-71.)

Key words: Multifetal pregnancy reduction, embryos, ethics

In 1988, the Ethics Committee of the American Fertility Society published its reaction to the Roman Catholic *Instruction*, which condemned, *inter alia*, both the destruction of fertilized eggs from the moment the zygote has formed and the reduction of multifetal pregnancies. The Ethics Committee published a more comprehensive statement in 1990.) We present here a report on a number of recent responsa (rabbinic rulings) on these two issues, which have initiated the discussion for contemporary Jewish scholars addressing the matter.

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The Jewish "magisterium" consists of the Bible, the Mishnah and Talmud, and universally accepted codifications such as Maimonides' Mishneh Torah or the later Shulhan Arukh. These are the sources for the investigation of the traditional Jewish position on any ethical or legal issue. Rulings on contemporary issues cannot be promulgated by any central authority, there being no formal hierarchical structure to the various contemporary rabbinic authorities and courts. Positions on contemporary issues are developed by circulation of responsa to questions posed to various rabbinic authorities. Collegial review and community acceptance eventually allow for specific opinions to emerge as dominant. (However, even when one view surfaces as authoritative, individual rabbis or laymen will often defer to their local authority whose position is considered decisive.) Not all authorities of Halakhah (traditional Jewish law) allow in vitro fertilization, even if the husband is the sperm donor.4-6 The responsa quoted here assume the questioner has accepted the lenient opinions permitting in vitro fertilization.

Status of embryo

One need not look past the famous Mishnah allowing for abortion of a fetus to realize that traditional Jewish ethics and law differentiates between an unborn fetus and a born human being. The Mishnah rules that "[if] a woman is in hard labor, they chop up the child in her womb and they remove it limb by limb, because her life takes precedence over its life. [If] its greater part had gone forth, they do not touch it, for they do not set aside one life on account of another life."7

Maimonides8 codifies this as law, declaring the fetus in such a case to be a rodef, an agressor pursuing another to kill him or her and who, according to Jewish law, must be killed rather than allowed to accomplish the murder. But if the baby's head has emerged, it attains human status and may not be touched, he continues, "for one may not put aside one life for the sake of another. This is the way of the world." This distinction between an unborn fetus and a born human being is rejected by the Roman Catholic Instruction, which reasserts the position that "from the time the ovum is fertilized, a new life is begun which is neither that of the father nor of the mother; it is rather the life of a new human being Right from fertilization is begun the adventure of a human Life 2

Within Jewish law, there are many situations in which a pregnancy may be terminated, although abortion on demand is repulsive to the ethics of the Halakhah. (Bleich⁹ has summarized the extensive halakic literature on the subject.) Still, nontransplanted embryos fertilized artificially in vitro have no standing as fetuses in Jewish law. In a recently published responsum, Haim David Halevi,10 Chief Rabbi of Tel Aviv, rules that "all eggs fertilized in vitro have no standing as embryos...and one may discard them if they were not chosen for implanting, as the law of abortion applies only to [procedures in] the womb But in vitro, as was said, there is no prohibition at all."

A similar ruling is offered by Mordechai Eliyahu,11 Chief Rabbi of Israel, who writes that "all fertilized eggs which are destined to be implanted in the mother's womb should not be destroyed, as a live fetus will yet develop from them. But those eggs which have not been chosen for implantation may be discarded." Neither authority offers any detailed analysis of his legal ruling, apparently considering the position to be obvious and noncontroversial from the perspective of Jewish law and ethics. Indeed, as Bleich¹² points out in his recent summary of halakhic attitudes toward fetal research, an aborted fetus in the early stages of gestation does not require burial.

It is not unreasonable to assume that to some extent these rulings are based on the Talmudic statement that an embryo is "mere water" within the first 40 days of conception and that it is not viable in vitro.13 If the embryo could be maintained artificially outside of the womb until much later stages of gestation, it is not clear if these rulings would apply.

Related ethical issues

These rulings have interesting implications for a number of other issues. Bleich12 has noted that the tissue of a spontaneously aborted fetus may be used for research purposes if there is a reasonable basis for assuming that practical medical benefits will ensue within a reasonable time period (the specific details of which require individual analysis). One might well argue that untransplanted embryos, which never had viable status, might be used for the purpose of research before they are discarded.

One must keep in mind, though, that those rabbinic authorities who allow in vitro fertilization do not consider the procurement of the sperm to be a "wasting of seed" because the procedure is used to overcome existing problems of infertility. It is not at all clear that rabbinic approval would be forthcoming for in vitro fertilization solely for the purpose of research. Likewise, it is not certain that properly obtained semen may be used to fertilize more eggs than would be necessary to assure a sufficient number of embryos for transplantation to obtain "excess" untransplanted embryos that would be used for research purposes. Nevertheless, "Jewish law does not posit . . . an exclusionary rule that would, post factum, preclude the use of illicitly obtained tissue for an otherwise sanctioned purpose" such as medical research.12

We may also surmise that the embryos may be examined for the purpose of sex selection. The technology to do this is already available.14 The primary halakhic concern is not with the decision to engage in sex preselection but with the method to be used in effecting sex determination.15 For example, aborting a fetus that is not of the desired sex is unequivocally prohibited, but if in vitro fertilization is used to overcome existing problems of infertility and only some of the embryos will be transplanted, it would seem that selecting an embryo of the desired sex would be allowed.

Similarly, screening embryos for genetic defects would be permitted. However, permitting in vitro fertilization solely for the purpose of genetic screening rather than for overcoming a fertility problem awaits a further, more complete analysis.

Multifetal pregnancy reduction

The issue of fetal reduction is more problematic. Increased use of ovulatory drugs and related therapies has yielded an increasing number of multifetal pregnancies. As a result of the premature births associated with such pregnancies, these fetuses are in significant jeopardy. Artificial reduction of the number of fetuses has been suggested as a method of reducing this risk, yet each individual fetus has standing in Jewish law, albeit not as a full person. There being no blanket permission for abortion, a more detailed justification of any ruling allowing multifetal pregnancy reduction is required.

As the Committee on Ethics of the American College of Obstetricians and Gynecologists¹⁶ takes note, a distinction may be made between multifetal pregnancy reduction and selective termination of an anomalous fetus. "Insofar as the intention of selective termination is different from that of other multiple fetal reductions, its ethical rationale is importantly distinctive. That is, the intention in selective termination is to avoid having a child with a known medical problem, whereas the intention in multifetal reduction is to prevent problems that are secondary to multifetal gestation and premature birth."

The question of multifetal pregnancy reduction was recently taken up by Yitzhak Zilberstein¹⁷ in a responsum to the Israeli Medical-Halakah Group. If the mother's life is in danger, he writes, each fetus is a *rodef* and can be killed to save the mother, as noted above. But if the danger is to the various fetuses and not to the mother, each fetus is an aggressor and victim with equal status; it therefore might not be permissible to put aside one soul for the sake of another.

Searching for a legal analogy for this situation, Zilberstein focuses on the case of a group of people who are in mortal danger and who can be saved by sacrificing one innocent member of the company. He notes that most halakhic authorities agree that in such cases all must allow themselves to die rather than themselves give up an innocent person. However, he finds a number of authorities who limit these rulings to cases in which in theory the innocent person who would be sacrificed might have been able to escape. If it were absolutely certain that all would be lost unless one were forfeited, these authorities would allow some innocent people to be selected by lottery and sacrified to save the others.

These conclusions apply to cases concerning full humans who have standing as viable persons. However, Zilberstein continues, our case concerns fetuses, all of which are already condemned to death. Multifetal pregnancy reduction should therefore be allowed, he ruled, noting that this might then be a case of "fetal life-saving" rather than "fetal reduction." (As Berkowitz and Lynch¹⁸ have noted, the term chosen to describe a procedure such as this is significant.) Shlomo Zalman Auerbach, one of the leading contemporary rabbinic

authorities in Jerusalem, is quoted elsewhere as also tending to allow the procedure to save the remaining fetuses.¹⁹

Zilberstein's decision seems to assume that without multifetal pregnancy reduction none of the fetuses would survive the multiple pregnancy. This suggests that if the medical indication is that they would survive, albeit with serious physical and/or mental deficiencies, multifetal pregnancy reduction might be ethically prohibited. However, Halevi¹⁰ also allows the procedure without presuming that all were otherwise doomed to death. He notes that there is no unanimity of opinion among halakhic authorities concerning abortion, with some taking a most restrictive position and others allowing abortion of Tay-Sachs fetuses even in the third trimester. As the vast majority hold that the abortion of nonviable fetuses is not homicide at all, he feels that a lenient position should be maintained because without reduction the fetuses most probably would be born prematurely and with serious physical and/or mental disabilities. He10 therefore allows reducing the pregnancy to the extent necessary to ensure that the remaining fetuses would be born healthy and whole.

Eliyahu¹¹ also allows the procedure, adding that the reduction could be done at any stage of the pregnancy, although it is better to do it as early as possible, preferably within the first 40 days, the period for which halakhists take the most liberal position on abortion. It would therefore be preferable, from this perspective, to use transvaginal sac aspiration rather than intracardiac potassium chloride administration via the transabdominal route, because the former may be done at an earlier stage of pregnancy.²⁰

Zilberstein, Halevi, and Eliyahu all maintain that the number of fetuses to be destroyed is a medical question and should be decided by the doctors involved, who must determine the minimum number that need to be killed to ensure a good prognosis for the mother and remaining fetuses. (Auerbach is not quoted on the issue.) This puts an ethical burden on the medical professional to be completely current on the statistical and medical studies associated with multifetal pregnancy outcome and multifetal pregnancy reduction, so that the absolute minimal number of fetuses to be killed could be determined reasonably. No halakhic authority suggests multifetal pregnancy reduction for convenience or choice, such as reducing twins to singletons.

Comment

Jewish law and ethics disagrees with the *Instruction*'s position that "no moral distinction is considered between zygotes, pre-embryos, embryos or fetuses." It maintains that eggs that have been fertilized in vitro and that have not yet been transplanted have no stand-

ing and may be discarded. Multifetal pregnancy reduction is a morally acceptable procedure, although care must be taken to kill the minimum number of fetuses that will reasonably assure that the remaining fetuses will be born healthy and whole.

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Clinical Articles

Should anticardiolipin tests be performed in otherwise healthy pregnant women?

E. Nigel Harris, MPhil, MD, DM, and Joseph A. Spinnato, MDb Louisville, Kentucky

Positive anticardiolipin and lupus anticoagulant tests are not confined to patients with the antiphospholipid syndrome, and the usefulness of these tests in healthy pregnant women is uncertain. This study sought to determine the prevalence of anticardiolipin antibodies and correlation with pregnancy outcome in 1449 pregnant women. Results were compared with 40 patients with the antiphospholipid syndrome. Persistence of positive anticardiolipin antibody tests was also ascertained. Twenty-six of 1449 sera (1.79%) were immunoglobulin G anticardiolipin positive, and 63 (4.3%) were immunoglobulin M anticardiolipin positive. Twenty-three of 26 positive for immunoglobulin G anticardiolipin and 55 of 63 positive for immunoglobulin M anticardiolipin results were low. Anticardiolipin positivity did not correlate with complications or outcome. Immunoglobulin G isotype and level distinguished patients with antiphospholipid syndrome from otherwise healthy women with positive anticardiolipin tests. In healthy pregnant women positive anticardiolipin tests occur infrequently, at low levels, and are rarely associated with adverse pregnancy outcome. This test should be requested only when the antiphospholipid syndrome is suspected. (AM J OBSTET GYNECOL 1991;165:1272-7.)

Key words: Anticardiolipin, lupus anticoagulant, antiphospholipid syndrome, pregnancy loss, systemic lupus erythematosus

Antibodies that bind phospholipids are detected by one of three tests: lupus anticoagulant, anticardiolipin, or Venereal Disease Research Laboratory tests.1 These antibodies have been associated in some, but not all, studies with recurrent pregnancy loss, arterial or venous thrombosis, and thrombocytopenia. Of the three tests, the anticardiolipin test is accepted generally as the most sensitive, the easiest to obtain, and the subject of most recent efforts to standardize; hence, it has been widely adopted by university and commercial laboratories. In spite of this, the association of anticardiolipin antibodies with fetal loss has been the subject of much controversy.²⁻⁶ Physicians are often uncertain whether this test should be ordered in otherwise healthy pregnant women and what action should be taken if a pregnant woman is found to have a positive test. The latter question is of vital importance, because affected women with recurrent pregnancy losses have been reported to have successful pregnancy outcome after treatment with prednisone, heparin, or high-dose immunoglobulin. These treatment modalities may be efficacious but have a high risk of side effects or, in the case of immunoglobulin therapy, may be expensive. Hence, the decision to treat should be based on clear guidelines.

Recently some investigators have examined anticardiolipin tests prospectively in otherwise healthy populations of pregnant women.^{8,9} One study reported an association of positive test results with low-birth-weight babies and spontaneous abortion,⁸ and another found an association with spontaneous abortion in those few women who had moderate or high immunoglobulin G (IgG) anticardiolipin levels.⁹

This report is a survey of sera for anticardiolipin antibodies in 1449 women admitted to the obstetric wards of a local hospital, to determine the degree to which positive anticardiolipin tests are associated with pregnancy outcome and complications. We compared the results of our study population with a group of 40 patients with the antiphospholipid syndrome.

Methods

All women admitted to the obstetric wards of the Alliant Health Care System (Louisville, Ky.) between

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21

1

9

53

NS

NS

NS

NS

		Race			
Isotype	Level (semiquantitative)*	White (n = 1229)	Nonwhite (n = 228)	Total No.	Significance $(p < 0.05)$
IgG	High	0	0	0	NS
J	Medium	3	0	3	NS

6

0

2

Table I. Positive anticardiolipin test results grouped according to isotype, level (semiquantitative), and race

NS, Not significant.

IgM

Low

High

Low

Medium

15

1

7 44

May 15, 1989, and March 15, 1990, were told of the study, and permission was sought to have their blood tested for anticardiolipin antibodies at the time blood was being drawn for other routine tests. A total of 1449 women consented to participate in the study. Most of the patients were at or near term ($\pm 90\%$). While patients with late second-trimester and early third-trimester complications were actively enrolled in the study, such patients were few. After consent was obtained, the admitting nurse filled in a form with the patient's name, age, race, and specified pregnancy complications. Pregnancy complications specified were preeclampsia, eclampsia, chronic hypertension, insulin-dependent diabetes mellitus, gestational diabetes, intrauterine growth retardation, abruptio placentae, stillbirth, and preterm labor. There was a category on the form for other complications to be specified. If the pregnancy was not associated with any complications, the nurse entered "none." The frequency of use of aspirin, corticosteroids, or other immunosuppressive therapy was not determined and was assumed to be low.

Sera from 40 patients with the antiphospholipid syndrome were analyzed for anticardiolipin antibodies. These patients were defined as having the antiphospholipid syndrome on the basis of a history of one or more episodes of venous or arterial thrombosis, recurrent abortion or fetal death, or thrombocytopenia associated with a medium- or high-positive anticardiolipin test result or a positive lupus anticoagulant test.1 These test results should have been positive on more than one occasion at least 8 weeks apart.1

The anticardiolipin assay was performed with an enzyme-linked immunosorbent assay method.4 Five milliliters of a clotted blood specimen was obtained from each patient. This specimen was centrifuged for 15 minutes and the sample was stored at -20° C until anticardiolipin measurement was performed. All patients' sera were run at a dilution of 1:50 in duplicate on three different days and the mean level was determined. Samples were analyzed for IgG and IgM anti-

cardiolipin and antibody levels reported in GPL units (for IgG) and MPL units (for IgM).10 Anticardiolipin levels for unknown samples were computed from a log-logistic calibration curve obtained by running five calibration standards on each enzyme-linked immunosorbent assay plate. On the basis of analysis of several thousand normal sera in the past, an abnormal IgG anticardiolipin result was taken as a level >5 GPL units, and an abnormal IgM result was taken as a level >5 MPL units. In addition to reporting antibody levels in GPL or MPL units, a semiquantitative measurement was used11: low, 5 to 20 GPL units (IgG) or 5 to 20 MPL units (IgM); moderate, 20 to 80 GPL units or 20 to 80 MPL units; and high, >80 GPL units or >80 MPL units. The latter semiquantitative designation has enabled better comparison of anticardiolipin results between laboratories.11 The lupus anticoagulant and other antiphospholipids were not assessed in this study.

The frequency of positive anticardiolipin tests between and among subsets of the study group was evaluated with x2 analysis and Fisher's exact test, where appropriate. Significance level was set at $\alpha = 0.05$.

Results

Sera were obtained from 1449 women. Two hundred twenty-eight were nonwhite, and 1221 were white. The mean age of white patients was 27 ± 5 years, and the mean age of nonwhite patients was 23 \pm 5 years. With the exception of stillbirth, there was no statistical difference in the frequency of complications according to race. In spite of its infrequent occurrence, stillbirth (white, 1; black, 3) was statistically more likely among nonwhite patients (p = 0.027). However, anticardiolipin antibodies were not observed among these patients.

Twenty-six (1.79%) of the 1449 serum specimens analyzed were positive for IgG anticardiolipin and 63 (4.3%) were positive for IgM anticardiolipin. These results, defined semiquantitatively-high, medium, low,

^{*}For IgG anticardiolipin, high, >80 GPL units, medium, 20 to 80 GPL units; and low, 5 to 20 GPL units. 12.13 For IgM anticardiolipin, high, >80 MPL units; medium, 20 to 80 MPL units; and low, 5 to 20 MPL units. 12.13

Complication	Anticardiolipin negative	Anticardiolipin positive							
		IgG			IgM			Total	Total anticardiolipin
		Low	Medium	High	Low	Medium	High	No.	positive (%)
Preeclampsia	58	1	0	0	3	0	0	62	6.4
Eclampsia	3	0	0	. 0	0	0	0	3	0
Chronic hypertension	15	0	0	0	0	0	0	15	0
Insulin-dependent diabetes mellitus	6	0	0	0	0	0	0	6	0
Gestational diabetes	17	0	0	0	0	0	0	17	0
Abruptio placentae	3	0	0	0	1	0	0	1	33
Intrauterine growth retardation	23	0	0	0	0	0	0	23	0
Stillbirth	4	0	0	0	0	0	0	4	0
Preterm labor	23	. 0	0	0	0	0	0	23	0
Other	174	2	0	0	3	2	0	181	3.9

0

46

Table II. Pregnancy complications and outcome according to anticardiolipin test result

18

3

or normal-and classified according to race, are shown in Table I. There was no difference in results according to race. Of the 26 patients with positive IgG anticardiolipin results, 23 were low positive, 3 medium positive and none high positive (Table I). One of the three patients with medium-positive IgG anticardiolipin was already known to us as having the antiphospholipid syndrome (four previous pregnancy losses and multiple deep venous thromboses). The result obtained in this patient was the lowest ever recorded for the 2 years we had been monitoring her. (She was being treated with high-dose prednisone and intermittent immunoglobulin infusions during that pregnancy, which ended in a live but premature birth.) Of the 65 patients with positive IgM anticardiolipin results, 55 were low positive, nine medium positive, and one high positive. The one patient with a high-positive IgM anticardiolipin result also had medium-positive IgG anticardiolipin and was known to have a false-positive Venereal Disease Research Laboratory test result but had no underlying disease. Pregnancy outcome was normal.

1053

None

The overwhelming majority of patients with positive anticardiolipin tests had no complications during pregnancy. Thus 21 of 23 (91.3%) patients with positive IgG anticardiolipin test results and 54 of 63 (76%) patients with positive IgM anticardiolipin results had no pregnancy complications (Table II). Of the three women with medium-positive IgG anticardiolipin results, all had live births, although the one with the antiphospholipid syndrome (as discussed) was treated during pregnancy.

In women with specified pregnancy complications, few had positive anticardiolipin test results, and when these were positive, levels were low. Thus, of the 62 women with preeclampsia, only four had positive anticardiolipin tests, three were low IgM positive and one was low IgG positive. This frequency (4/62) was not

significantly different from that of patients with other complications (7/181) or patients who had no complications (75/1148). The only other complication categories in which test results were positive were abruptio placentae (one low-positive IgM) and seven in the "other" category (Table II).

1148

6.5

Anticardiolipin levels according to isotype in the study population are compared with level and isotype in 40 patients with the antiphospholipid syndrome (Figs. 1 and 2). IgG anticardiolipin levels in most patients with the antiphospholipid syndrome were much higher than any of the IgG anticardiolipin—positive levels in the study population (Fig. 1). Only 20 of the 40 patients with antiphospholipid syndrome had positive IgM anticardiolipin tests and only six of 20 had levels >20 MPL units. In the study population 63 of 1449 patients had positive results, and there were nine patients with levels >20 MPL units (Fig. 2). Thus there was considerable overlap in IgM anticardiolipin levels between the patients with antiphospholipid syndrome and the study patients.

In an effort to determine whether women in the study population might have underlying disorders contributing to positive anticardiolipin tests, e.g., systemic lupus erythematosus or antiphospholipid syndrome, and to determine how long positive test results persisted, we invited all 99 patients with positive test results to return for a more detailed history and repeat anticardiolipin test. Twenty-seven patients returned for a follow-up interview within about 3 months of completion of pregnancy. The patient known to us with the antiphospholipid syndrome is not included in this analysis.

Twenty-three of the 27 patients who returned had had uncomplicated pregnancies. Of the remaining four, one had preeclampsia, one had abruptio placentae, and two had postdate births. On questioning, none

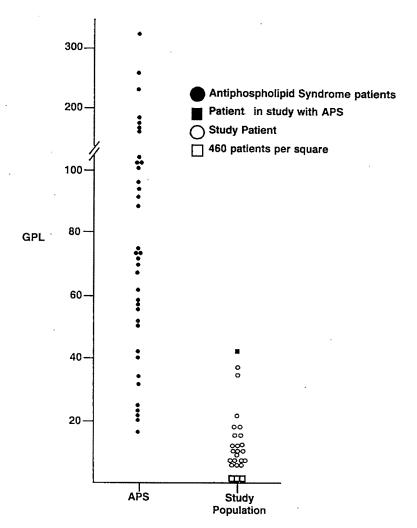


Fig. 1. Comparison of IgG anticardiolipin levels in 40 patients wth antiphospholipid syndrome (APS) with those of 1449 pregnant women in study. Only a minority of pregnant women had positive IgG anticardiolipin results and these were almost always lower than levels in patients with antiphospholipid syndrome. The highest value in study population (43 GPL units) was in a patient with the antiphospholipid syndrome being treated with prednisone and intermittent Ig therapy. This was the lowest result she had ever achieved, and she gave birth to a live baby at 32 weeks' gestation.

of these patients had symptoms of antiphospholipid syndrome, systemic lupus erythematosus, or other autoimmune diseases. Nine of the 26 had a history of abortion, five having one, two having two, one having three, and one having four previous abortions. All patients with histories of abortions had low-positive IgM anticardiolipin results, as did the majority of patients who were screened in this study. Surprisingly, on retesting, 22 of 27 patients remained with positive results at levels comparable with their original values.

Comment

Recent studies have screened healthy pregnant women for anticardiolipin antibodies and sought prospectively to compare positive anticardiolipin test results with outcome. The study by Lockwood et al.8 is

the best known; in their survey of 737 healthy women, they found an association between positive anticardiolipin test results and low-birth weight babies or spontaneous abortion. However, the anticardiolipin test result was infrequently positive in these women, and it appeared that the majority of women tested had low anticardiolipin levels although measurement of levels did not correspond to currently accepted criteria. 10, 11 A second study by Brown et al.9 of 1200 women found only 15 (1.25%) to have positive IgG anticardiolipin tests. Six had moderate- to high-positive IgG anticardiolipin tests, and three of these had spontaneous abortion. They found spontaneous abortion to be no more frequent in women with low-positive test results than in 393 women with negative test results.

We have surveyed sera of 1449 pregnant women at

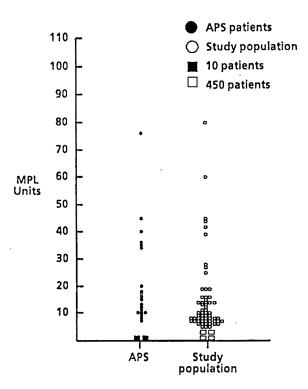


Fig. 2. Comparison of IgM anticardiolipin levels in 40 patients with antiphospholipid syndrome (APS) with those of 1449 pregnant women in study. In contrast to IgG anticardiolipin (Fig. 1), only a minority of antiphospholipid syndrome patients had positive IgM anticardiolipin results, and there was considerable overlap between levels in this group and those of the study population.

or near the time of delivery for IgG and IgM anticardiolipin results and have sought to determine the prevalence of positive test results in these women and the degree to which these tests correlate with pregnancy outcome. The anticardiolipin test used to screen sera utilizes several of the improvements in enzyme-linked immunosorbent assay techniques and units of measurement that have been recommended by two international workshops designed to standardize the anticardiolipin test^{10,11}; hence, we believe our findings can be reproduced in other laboratories.

We found that the prevalence of positive anticardiolipin tests in a population of pregnant women at term is low. Only 1.79% (26/1449) had positive IgG anticardiolipin tests and 4.3% (63 of 1449) had positive IgM anticardiolipin. These prevalences are very similar to those of surveys of large numbers of nonpregnant blood donors (unpublished data) and suggest that normal pregnancies do not induce autoantibody production—a conclusion reached by other investigators. ¹³⁻¹⁵ More importantly, of the 26 patients with positive IgG anticardiolipin results, only four had moderate levels (none were high), and the patient with the highest level was already diagnosed as having the antiphospholipid

syndrome. The infrequency of pregnancies with second-trimester or early third-trimester complications and delivery in this study limits conclusions to be drawn for such patients.

It was noteworthy that when IgG anticardiolipin levels of the pregnant women with positive test results were compared with levels in patients with antiphospholipid syndrome, levels in the latter were higher than those in the former. It was just as important that only a small number of patients with the antiphospholipid syndrome had elevated IgM anticardiolipin levels, and there was considerable overlap of these levels with levels in the pregnant women. Few women with low-positive results had adverse pregnancy outcome; therefore the findings of this study reemphasize findings in some16-19 but not other studies.8, 15 We can only conclude that IgG anticardiolipin results at low levels and IgM anticardiolipin results at low and probably moderate levels are infrequently associated with adverse pregnancy outcome.

The fact that only a minority of women with positive results had adverse pregnancy outcomes identifies a dilemma faced by several other past studies of anticardiolipin antibodies. When populations of pregnant patients or patients with disorders such as systemic lupus erythematosus are screened, the overwhelming majority of positive tests are usually low positive. Recurrent pregnancy losses may be infrequent in these women, although they have positive anticardiolipin test results. Investigators finding these results in patients with systemic lupus erythematosus often conclude that there is no association with recurrent pregnancy losses.20 In fact, the association could well have been missed⁵ because recurrent fetal loss may have occurred only in the infrequent patient with high anticardiolipin levels. (One author, E.N.H., has surveyed sera of patients with systemic lupus erythematosus from a number of institutions in which very few or no serum samples have had anticardiolipin levels in ranges seen in the antiphospholipid syndrome; such series are unlikely to find associations between anticardiolipin antibodies and fetal loss.)

Surprisingly, the positive anticardiolipin results of a majority of patients remained positive for periods at least up to 3 months post partum, although none had clinical features of the antiphospholipid syndrome or systemic lupus erythematosus. One patient (with the highest IgM anticardiolipin level) was known to have a false-positive test result for syphilis, but she had no symptoms of an autoimmune disorder and her two pregnancies were without complications. This finding leads us to conclude that persistently positive tests need not be confined to patients with the antiphospholipid syndrome or systemic lupus erythematosus but may persist for long periods even in the absence of clinically

apparent autoimmune disorders. Perhaps it is in this population of patients with low positive results that abortions occur more frequently, the subpopulations with subclinical autoimmune disease.21-28 The persistence of those antibodies long after pregnancy suggests that the pregnant state did not induce autoantibody production.

On the basis of the above data, we recommend that the anticardiolipin test not be done to screen otherwise healthy women. The test is positive infrequently in this population, and, even when positive, levels are usually low and of uncertain significance. We suggest that aggressive treatment during pregnancy of women with histories of recurrent abortion who have low-positive IgG anticardiolipin or low- or moderate-positive IgM anticardiolipin results may not be justified. The latter statement is made because, in this study of largely healthy women, IgG or IgM anticardiolipin results at levels just cited were rarely associated with adverse pregnancy outcome. This does not exclude the possibility, however, that a low-positive anticardiolipin test result plus other, yet to be identified factors can combine to cause pregnancy loss and that this adverse combination can be ameliorated with immunosuppressive therapy. However, the latter postulate provides little basis for aggressive treatment. Finally, we recommend that the anticardiolipin test be ordered primarily when there is a high index of suspicion, on the basis of clinical presentation, that a patient has the antiphospholipid syndrome. Women with histories of unexplained pregnancy loss (particularly if this occurred in the second or third trimester), deep vein thrombosis, or stroke and all pregnant women with systemic lupus erythematosus should be screened for anticardiolipin antibodies. 12, 23 Treatment should be considered if the IgG anticardiolipin test result is moderate to high positive or the lupus anticoagulant test result is positive.

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The effect of maternal cocaine use on the fetus: Changes in antepartum fetal heart rate tracings

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Fetal heart rate tracings of pregnancies complicated by cocaine use were analyzed to evaluate the effects of subacute maternal cocaine use on the fetus. Nonstress tests were performed twice weekly on patients from the perinatal substance abuse clinic with screening of maternal urine samples for cocaine, amphetamines, phencyclidine, and opiates at the time of each examination. Nonstress tests performed when the urine toxicology screen was positive for cocaine alone (positive cocaine nonstress tests) were paired with those from the same patient but performed when the screen was negative (negative cocaine nonstress tests). The nonstress tests were analyzed with the Lyons scoring system, which evaluates the baseline heart rate, the oscillatory amplitude of the baseline, the oscillatory frequency, decelerations, and accelerations. Twenty pairs of nonstress tests from 20 patients were analyzed. The total score was higher on the negative cocaine nonstress test in 70% of the pairs and equal in the remaining 30% (p < 0.001). Significant differences occurred in the oscillatory amplitude (p < 0.001), frequency (p = 0.002), and acceleration scores (p = 0.03) but not the fetal heart rate baseline or the deceleration scores. The observed changes may reflect alterations in fetal central nervous system neurotransmitters and fetal state regulation, which may affect the developing central nervous system of cocaine-exposed fetuses and in turn play a role in the developmental and behavioral abnormalities observed in cocaine-exposed infants. (AM J OBSTET GYNECOL 1991;165:1278-81.)

Key words: Cocaine, nonstress test, fetal behavioral states

Cocaine, a potent local anesthetic, inhibits the presynaptic uptake of neurotransmitters such as norepinephrine and dopamine, resulting in a relative excess of these agents in the synaptic cleft. The most notable physical manifestations, tachycardia, arrhythmias, peripheral vasoconstriction, and hypertension, result from cardiovascular stimulation caused by this neurotransmitter excess.2 The euphoric and addictive qualities of cocaine are thought to be secondary to the effects on central nervous system neurotransmitters.3 Long-term cocaine use during pregnancy has been associated with numerous adverse perinatal outcomes, such as intrauterine growth retardation, preterm delivery, abruptio placentae, and congenital anomalies. 4-6 The presumed etiology is repetitive hypoxic insults to the fetus and the placenta as a result of cocaine-induced uterine artery vasoconstriction.5,7,8 Whereas numerous studies have dealt with the effects of long-term cocaine use during pregnancy, few clinical investigations have directly examined the short-term effects of maternal cocaine use on the fetus. To address this question, we analyzed the fetal heart rate (FHR) tracings of pregnancies complicated by cocaine use.

Material and methods

Nonstress tests (NSTs) were performed twice weekly on patients from the Harbor-University of California, Los Angeles, Medical Center perinatal substance abuse clinic beginning at 30 weeks' gestation, as part of the clinic protocol. Maternal heart rate and blood pressure were measured with the patient in the left lateral position at the beginning of the examination. The fetal acoustic stimulation test, with a model 5C electronic artificial larynx (Western Electric, New York), as described by Smith et al.,10 was performed if no spontaneous accelerations occurred within the first 5 to 10 minutes. Maternal urine samples were obtained at the time of each examination and were screened for cocaine, amphetamines, phencyclidine, and opiates with the enzyme multiplied immunoassay technique (E.M.I.T. D.A.U. assay, Syva, Palo Alto, Calif.). NSTs performed when the urine toxicology screen was positive for cocaine alone were designated as positive cocaine NSTs, and those performed when the screen was negative were designated as negative cocaine NSTs. The first positive cocaine NST obtained was paired with the closest chronologically available negative cocaine

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Table I. Lyons scoring system for evaluation of antepartum FHR tracings

	Score							
Parameter	0	1	2					
Baseline (beats/min)	<100 and >180	100-120 160-180	120-160					
Oscillatory amplitude (beats/min)	≤5	$>$ 5 and \leq 10 or \geq 25	>10 and <25					
Oscillatory frequency (cycles/min)	<2	≥ 2 and ≤ 4	>4					
Decelerations	Late ≥25% of uterine contractions	Late <25% of uterine contractions	None					
Accelerations	Marked variable None No spontaneous	Mild to moderate variable <4/20 min	≥4/20 min					

Modified from Lyons et al. A scoring system for nonstressed antepartum fetal heart rate monitoring. AM J OBSTET GYNECOL 1979;133:242.

Table II. Cardiovascular parameters

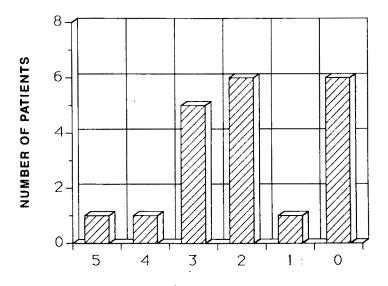
	Negative cocaine NST	Positive cocaine NST
No.	20	20
Maternal heart rate (beats/min)	79.1 ± 1.5	80.8 ± 2.1
Maternal mean arterial pressure	73.5 ± 1.2	67.9 ± 3.0
FHR (beats/min)	133.7 ± 1.8	134.6 ± 1.7

NST from the same patient. Each NST was graded with the Lyons scoring system (Table I) by one of the authors (B.L.T.), who was blinded to the identity of the patient and the results of the screen. The Lyons scoring system assigns numeric values (0, 1, or 2) to five parameters on the NST: the baseline heart rate, the oscillatory amplitude of the baseline, the oscillatory frequency, decelerations, and accelerations, with the lower scores assigned to the more abnormal findings.11 The scores for the paired NSTs were compared for both the total and the individual parameters. Gestational age and cardiovascular data are presented as mean ± SEM. Statistical analysis of the paired scores was performed with the Wilcoxon signed-rank test and McNemar's test of symmetry. Cardiovascular and gestational age differences were analyzed with the Student paired t test. Statistical significance was accepted with p < 0.05.

Results

Twenty pairs of NSTs from 20 patients were analyzed. There were no differences in maternal heart rate or blood pressure or in FHR between the positive and negative cocaine NSTs (Table II). There were no episodes of maternal tachycardia or hypertension and no fetal tachycardia in either group. The mean gestational age was 35.9 ± 0.6 and 36.1 ± 0.6 weeks for the negative and positive cocaine NST groups, respectively. The mean time between paired tests was 6.7 ± 1.1 days, with the negative cocaine NST occurring first in 11 pairs and the positive cocaine NST first in nine pairs. None of the patients had underlying medical illnesses, such as diabetes, hypertension, or collagen vascular disease, and none had preeclampsia, abruptio placentae, or intrauterine growth retardation or were delivered postdates (>42 weeks' gestational age).

The total score was higher on the negative cocaine NST in 70% of the pairs and equal in the remaining 30% (p < 0.001) (Fig. 1). When three points was used as a more clinically significant difference, indicating involvement of two or more parameters, 35% of the pairs had higher negative cocaine NST scores (p < 0.01). Among the individual parameters, significant differences occurred in the oscillatory amplitude (p < 0.001), frequency (p = 0.002), and acceleration scores (p = 0.03) but not the FHR baseline or the deceleration scores. Seventy percent of the pairs had higher amplitude scores on the negative cocaine NSTs, whereas higher frequency scores occurred on the negative cocaine NSTs in 55% of the pairs. None of the pairs had higher scores on the positive cocaine NSTs on either of these parameters. Thirty percent of the pairs had higher acceleration scores on the negative cocaine NSTs, whereas only 5% had a higher score on the positive cocaine NST. All three of these parameters were affected on the positive cocaine NST in 25% of



NUMBER OF POINTS NST SCORE WAS LOWERED WITH POSITIVE COCAINE SCREEN

Fig. 1. Magnitude of depression of Lyons NST scores associated with cocaine use. In 70% of patients NST score when urine toxicology screen was positive for cocaine was lower than when screen was negative (p < 0.001). In 35% of patients positive cocaine NST score was \geq 3 points lower than negative cocaine NST scores (p < 0.01).

the pairs, whereas none of the negative cocaine NSTs had all three variables involved (p < 0.05). Twenty percent of the positive cocaine NSTs were nonreactive, compared with none of the negative cocaine NSTs (p < 0.05).

Comment

The finding of lower scores on the NSTs obtained when the urine toxicology screens were positive for cocaine suggests that episodic cocaine use may have adverse consequences for the fetus. The differences observed between the positive and negative cocaine NSTs are probably not short-term hypoxic effects caused by uterine artery vasoconstriction. There were no discernible maternal or fetal cardiovascular changes (tachycardia or hypertension) at the time of the examination; therefore one would expect a similar absence of short-term effects on the uterine vasculature. The positive urine toxicology screens may represent use within the past 1 to 2 days, the period that cocaine metabolites are excreted in the urine and detectable by the present technology.12 If the patient were to be examined in closer proximity to the time of use, the maternal and fetal cardiovascular changes occurring with cocaine intoxication might be seen.

The changes seen in the positive cocaine NSTs, predominantly smaller, slower fluctuations of the baseline with fewer accelerations, are suggestive of the heart rate pattern seen during quiet sleep, or what has been described as fetal behavioral state 1F.¹³ Whereas the heart rate pattern is only one of the parameters used to characterize fetal states and there is some controversy whether true behavioral states exist before 36 to 38 weeks, the differences observed between the positive and the negative cocaine NSTs suggest cocaine-induced perturbations in FHR regulation, if not actual fetal state regulation.14 The regulation of fetal behavioral states is rather complex.15 Various areas in the central nervous system are involved and are sensitive to alterations in neurotransmitters such as serotonin, dopamine, and norepinephrine. Agonists or antagonists of these agents not only may acutely alter activity states but may cause prolonged disruption in state regulation. Clonidine, which blocks the presynaptic release of norepinephrine, has been shown to have immediate effects on electrocortical activity, fetal breathing, and heart rate variation and to cause rapid, irregular cycling of these activities for several hours after injection.16 Whereas cocaine results in the opposite effect on synaptic cleft norepinephrine levels, it too may have prolonged effects on the central nervous system centers responsible for state regulation. In addition, potent metabolites of cocaine, such as norcocaine, may be excreted into the amniotic fluid and recirculated in the fetal environment, causing central effects long after the initial maternal cocaine use.

Neonates exposed to cocaine in utero have been noted to have difficulties in behavioral state regulation and orientation, even if by history maternal use was limited to the first trimester.¹⁷ Hume et al.,⁹ using ul-

trasonography and FHR examination to investigate fetal state regulation, noted similar behavioral state disorganization in 65% of 20 cocaine-exposed fetuses followed up throughout the pregnancy, which correlated with difficulties in neonatal state regulation. Chasnoff et al.17 proposed that cocaine, by altering central nervous system neurotransmitters during embryologic development, behaves as a neurobehavioral teratogen. Because alterations in neurotransmitters during the sensitive stage of structural development in the first trimester probably will have some effect on later function, one cannot downplay the consequences of repetitive disruptions in the developing regulation of fetal behavioral states caused by cocaine use in the third

In summary, analysis of FHR tracings with and without recent exposure to cocaine demonstrates adverse changes with maternal cocaine use. These changes may reflect alterations in fetal central nervous system neurotransmitters and subsequently fetal state regulation, which may affect the developing central nervous system of cocaine-exposed fetuses. This may in turn play a role in the developmental and behavioral abnormalities observed in these infants. Further investigation may more fully characterize this relationship and provide insight into the normal regulation of fetal arousal states and the role that this plays in normal development.

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Qualitative evaluation of uterine contractions recorded by a double guard-ring tocodynamometer

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Qualitative characteristics of uterine contractions in various labors were evaluated by a double guard-ring tocodynamometer, according to the percentages of concurrent and synchronous contractions of two contraction curves when compared with overall contractile activity. For term and preterm labors, percentages of both concurrent and synchronous contractions were significantly higher than those of Braxton Hicks contractions; however, the concurrent and synchronous percentages of preterm labor were lower than those of term labor. Synchronous percentage in groups under maintenance therapy or active treatment in preterm labor were nearly the same; however, the percent concurrent under maintenance therapy was significantly lower than that during active treatment. Both concurrent and synchronous percentages were significantly higher during the active phase of labor than those during the latent phase. The percentages in the latent and active phases of spontaneous contractions were nearly the same; however, they increased significantly during the active phase of augmented contractions, as compared with the latent phase. When the percentages of concurrent and synchronous contractions at each cervical dilatation were retrospectively examined, both values increased significantly between 5 and 6 cm of cervical dilatation. The results suggest that this method can provide important information for understanding the quality of the contraction if attention is paid to the percentages of concurrent and synchronous contractions of two contraction curves and will aid in determining whether oxytocin or tocolysis should be given in term or preterm labor. (AM J OBSTET GYNECOL 1991;165:1282-6.)

Key words: Uterine contraction, guard-ring tocodynamometer, synchronization

The external tocography obtained from a guard-ring tocodynamometer has an advantage over the internal method for recording uterine contractions in obstetric practice because it does not require intrauterine manipulation and can therefore be easily used at any time, even in the second trimester of pregnancy. For these reasons, the external method is widely used for antepartum contraction monitoring, in the same manner as intrapartum monitoring, to judge preterm labor and the effect of tocolytic agents. Typically three patterns of contractions are observed: small contraction, the duration of which is ≤30 seconds; normal contraction wave, lasting about 60 seconds; and long contraction, lasting >3 minutes. Recently, we reported on the clinical features of the small contraction wave.1 In this report small waves were frequently observed when large contractions were suppressed by intravenous β_2 -stimulant infusion for the treatment of preterm labor.1 The long contraction wave that has been reported as resulting from hyperstimulation by oxytocics is sometimes

observed even in cardiotocography of normal pregnant women.2 However, the significance of the amplitude of contractions recorded by the guard-ring method in preterm or term labor has not yet been clarified. Therefore in a preliminary report we focused our attention on the synchronization of contractions at two points of the uterus recorded by small guard-ring tocodynamometers and attempted to elucidate other properties of contractions, in addition to duration and frequency, in routine obstetric practice.3 In that study the conduction of contractions between fundal and caudal regions of the uterus appeared to occur in both directions (i.e., downward or upward). Moreover, the synchronization of ascending points of the lower fifth, between baseline and peak point, of the contraction waves and of the peak points was significantly higher in the active phase than in the latent phase of labor.3

In this investigation, with a new device that can record two traces of fetal heart rate, fetal movements, and. two contraction curves simultaneously, we have attempted to evaluate quality differences of various contractions by monitoring two contraction curves recorded simultaneously in preterm or term labor.

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6/1/30966

Material and methods

One hundred twenty-four patients from 27 to 41 weeks of gestation were examined in our department at Saga Medical School from April 1988 to June 1989.

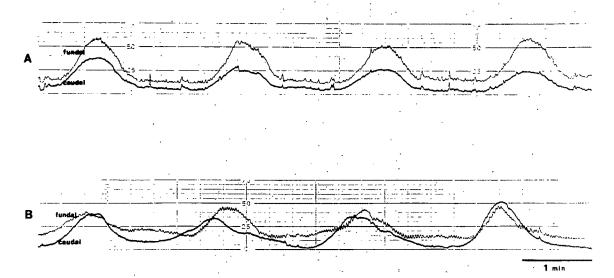


Fig. 1. Actual traces of uterine contractions recorded by double guard-ring tocodynamometer. *Thin line* was recorded from fundal part of uterus and *thich line* from caudal part. **A,** Typical synchronized contractions; **B,** asynchronous pattern.

The patients included 51 nulliparous and 73 multiparous women. Multiple gestations were not included in this study. Height and weight of the patients ranged from 145 to 173 (mean, 156.7) cm and 44.0 to 78.5 (mean, 59.4) kg, respectively. The period of gestation was calculated from the date of the last menstrual period and was confirmed with serial ultrasonographic measurements of crown-rump length and biparietal diameter. All cardiotocograms were obtained by the new device (Toitu Multiactocardiograph MT-430, Toitu Co. Ltd., Tokyo) attached with two small guard-ring tocodynamometers, 3.5 cm in diameter. The pregnant women were placed in the supine or semi-Fowler position and were fitted with the tocodynamometers at two different points on the uterus; one was attached to the middle part of the uterine fundus and the other was attached to the caudal part of the uterus at a distance of >15 cm. Two contraction curves were simultaneously recorded on the same sheet. Recording speed was 3 cm/min.

Contractions recorded in the cardiotocograms were divided into three groups, specifically, Braxton Hicks contractions and contractions in preterm or term labor. Braxton Hicks contractions were recorded from term uncomplicated gestations in patients before onset of labor. Threatened preterm labor was diagnosed by the onset of uterine contraction almost every 10 minutes as observed by external tocography in the outpatient department. Cases complicated with preterm labor were divided into two groups: patients receiving maintenance therapy who were treated with bed rest or oral administration of ritodrine hydrochloride (Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan) and those receiving active treatment with a drip infusion of rito-

Table I. Data on material and experimental conditions in this study

No. of cases	124
Before onset of labor	26
Spontaneous labor	41
Induction or augmentation	39
Preterm labor	- 18
No. of examined contractions	5,242
Measurable contractions	3,768
Not measurable contractions	1,474
Measurable contractions per examined contractions	0.72
Total recording time (min)	23,107
. Measurable period of time	17,482
Not measurable period	5,625
Measurable period of time per total	0.76
recording time	

drine. Term labors included spontaneous and augmented contractions. For the augmentation of labor, the titration method for oxytocin infusion was performed in all cases.

Actual records are shown in Fig. 1. A wave that elevated ≥ 2 mm from the baseline was considered as a contraction curve. Small waves, the duration of which was ≤ 30 seconds, were excluded from this study. Total contractions refers to the total number of contractions that were recognized, as mentioned subsequently. Contractions that appeared concurrently in upper and lower curves were counted as one, while lone contractions appearing in either upper or lower curve were also counted as one. The percent concurrent refers to the percent of total contractions that were recognized in the upper (fundal) and lower (caudal) curves simultaneously (Fig. 1, A and B). Contraction curves were considered to be synchronized when the peaks of two

Table II. Concurrent and synchronized percentages in various labors

	No.	Concurrent (%)	Synchronous (%)
Braxton Hicks contractions	116	53	12
Term labors	2874	88	41
Preterm labor	614	78	27
During maintenance therapy	121	52	23
During active treatment	493	85	28

For both concurrent and synchronous Braxton Hicks contractions versus preterm labor—during maintenance therapy and for synchronous preterm labor—during maintenance therapy versus during active treatment, p= not significant. For synchronous Braxton Hicks contractions versus all preterm labor, p<0.05. For synchronous Braxton Hicks contractions versus preterm labor—during active treatment, p<0.025. For all other comparisons, p<0.005.

Table III. Concurrent and synchronized percentages at each phase of term labor

	No.	Concurrent (%)	Synchronous (%)
All contractions	2874	84	41
Latent phase	666	81	30
Active phase	2208	90	44
Spontaneous contractions	1063	85	40
Latent phase	342	85	35
Active phase	721	85	43
Augmented contractions	1811	89	41
Latent phase	324	77	24
Active phase	1487	92	45

Concurrent: For all contractions, latent versus active phase, p < 0.005. For spontaneous versus augmented contractions, p < 0.005. For spontaneous contractions, latent versus active phase, p = not significant. For augmented contractions, latent versus active phase, p < 0.005. Synchronous: For all contractions, latent versus active phase, p < 0.005. For spontaneous, versus augmented, contractions, p = not significant. For spontaneous contractions, latent versus active phase, p < 0.05. For augmented contractions, latent versus active phase, p < 0.005.

such concurrent contractions occurred within 1 mm of each other (Fig. 1, A). Thus percent synchronous refers to the percent of total contractions in the upper and lower curves that were not only concurrent but also synchronized.

The χ^2 test was used to determine the significance of the results.

Results

Table I shows the material and experimental conditions of this experiment. Patients examined totaled 124 cases, including 26 cases before onset of labor, 41 of spontaneous labor, 39 of induction or augmentation cases, and 18 of preterm labor. Monitoring durations ranged from 41 to 243 minutes in each examination, depending on the patients' clinical conditions. In the examined records, about 70% of all contraction waves were accurately measurable (measurable contractions, 72%; measurable period of time, 76%) without intervention of the recording artifacts caused by maternal movements and tocodynamometer detachment, as indicated in the table. Table II shows concurrent and synchronous percentages of various labors. In term and preterm labors, both values were significantly higher than those of Braxton Hicks contractions; however, as shown in Table I, the concurrent and synchronized rates of preterm labor were lower than those of term labor. Preterm labor patients were then divided into two groups, specifically those receiving maintenance therapy and those receiving active treatment, as described in Material and methods. Synchronized values for both groups were nearly identical (23% vs 28%); however, the percent concurrent in patients receiving maintenance therapy was significantly lower than that of the active treatment group (52% vs 85%). Table III shows the concurrent and synchronous percentages for term labors. Both values in the active phase of labor were significantly higher than those in the latent phase. This change in percentages was not observed between the latent and active phases of spontaneous contractions (85% and 35% vs 85% and 43%); however, in augmented contractions both values increased significantly in the active phase as compared with the latent phase (latent phase, 77% and 24%; active phase, 92% and 45%). Considering these results, it can be said in general that concurrent and synchronous percentages increase in the active phase of labor as the labor progresses from the onset. Therefore both values at each dilatation of the cervix were retrospectively examined by comparing the contraction curves of the cardioto-

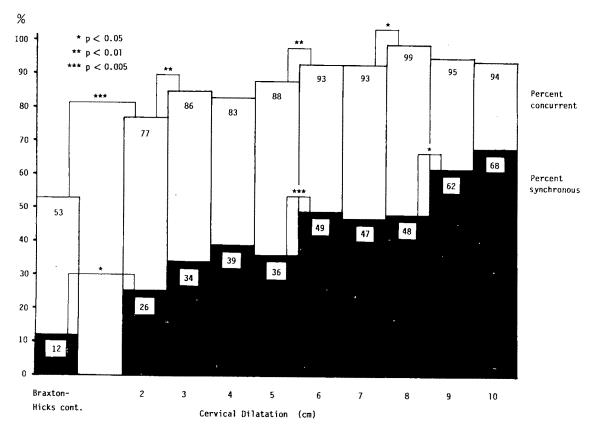


Fig. 2. Concurrent and synchronous percentages at each cervical dilatation in term labor. Statistical significances are represented in figure.

cogram with the corresponding time courses in the partogram of term labor, as shown in Fig. 2. Cervical dilatation was examined every hour and was done more frequently when the labor progressed at a faster rate. From three to 31 contractions were examined at each corresponding dilatation. Consequently, it was found that both values gradually increased with the progress of cervical dilatation. Furthermore, significant differences in the percent concurrent contractions were observed between 2 and 3 cm, 5 and 6 cm, and 7 and 8 cm. On the other hand, significant differences in the percent synchronous contraction were observed between 5 and 6 cm and 8 and 9 cm. Both values significantly increased between 5 and 6 cm of cervical dilatation.

Comment

The guard-ring tocodynamometer, which was invented by Smyth4 in 1957 to enable measurement of the intraamniotic pressure, is indirect and cannot quantitatively measure uterine pressure unless the specific conditions are set. Therefore the amplitude of the contraction curve cannot reveal the exact value of the force of the contraction and cannot be compared with other data. Thus we usually interpret the contraction data recorded by the guard-ring method through pattern recognition with frequency and duration. On the other hand, it is apparent that synchronization of contractions of the myometrial segments in the unicornuate human uterus is one of the most important factors in producing a strong expulsive force. Caldeyro et al.5 proposed these conditions as the optimal set of characteristics of uterine contractility: (1) large absolute intensity of the contractions; (2) strong fundal dominance; (3) good synchronization between the different parts of the uterus; (4) regularity in the rhythm, intensity, and form of the contractions; and (5) during the periods of relaxation, descent of the amniotic pressure to the level of normal tonus. In this study percent concurrent and percent synchronous contractions in term and preterm labor were significantly higher than those of Braxton Hicks contractions (Table II). It can be said that this result reveals the quality difference of each contraction, even if the shape of the contraction curve is the same. The fact that the differences were observed between the latent and active phases of labor (Table III), as reported in our previous article,⁸ also supports the quality difference of the labors. Moreover, the differences between the latent and active phases were more obviously observed in augmented contractions by oxytocin than in spontaneous labor, as shown in Table III. Considering the higher values of both percentages (85% concurrent and 35% synchronous) in the latent phase of spontaneous contractions, more efficient propagation mechanisms for excitation might already be prepared for at the time of spontaneous onset of labor. As for the whole course of delivery, both values gradually increased with the progress of cervical dilatation, as shown in Fig. 2; however, the contraction qualitatively changed between 5 and 6 cm, not between 3 and 4 cm of dilatation, which was described by Friedman⁶ as the turning point of the progress of labor. This result indicates that the turning point of characteristics of the contraction itself may exist between 5 and 6 cm of the cervical dilatation. Clarification of the fundamental mechanism of this change will prove difficult, but it may become an important factor in deciding whether oxytocin is used to augment contraction. In cases of preterm labor, both values were higher than those of Braxton Hicks contractions, especially the percent concurrent during active treatment (Table II). However, both values decreased to the levels of Braxton Hicks contractions, even though active tocolytic treatment was effective. According to these results, examination of both percentages may be useful in deciding whether tocolysis should be continued in cases of preterm labor.

The guard-ring tocodynamometer has many merits

and demerits for the recording of uterine contractions; however, this method can provide important information to understand the quality of the contraction if attention is paid to both concurrent and synchronous percentages, as presented in this study. More data are needed to improve this method in the future.

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A prospective, randomized comparison of the Pipelle endometrial sampling device with the Novak curette

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This prospective, randomized study compares sample adequacy, pain associated with endometrial biopsy, and correlation of endometrial histologic sampling with hysterectomy histologic results in specimens obtained by the Pipelle (Unimar Inc., Wilton, Conn.) device (N = 149) and the Novak (Miltex, Inc., Lake Success, N.Y.) curette (N = 126). During a 2-year period, patients with abnormal uterine bleeding seen in our ambulatory care facility were enrolled in this trial, unless they did not wish to participate or had a positive pregnancy test result. Patients in both groups were similar with respect to mean gravidity, parity, and menopausal status, although patients in the Novak group were older (43.4 vs 39.1 years, p = 0.005). Patients undergoing Novak biopsy had a mean pain score of 4.36 with 22 of 126 (17%) reporting severe pain, whereas patients undergoing Pipelle biopsy had a mean pain score of 3.21 with only 10 of 149 (6.7%) reporting severe pain (p < 0.05). These pain scores were not affected by menstrual day, gravidity, parity, or menopausal status. Insufficient tissue was reported in 12.8% of patients in the Pipelle group compared with 9.5% in the Novak group (p > 0.05). Fifty patients underwent subsequent hysterectomy. In 48 of 50 (96%), the pathologic results at hysterectomy were in agreement with the histologic findings at endometrial sampling. This clinical trial suggests that Pipelle biopsy appears to be as effective as the Novak curette in obtaining an adequate specimen for histologic analysis and is associated with less pain. (AM J OBSTET GYNECOL 1991;165:1287-9.)

Key words: Endometrial biopsy, Novak curette, Pipelle endometrial sampling device

Endometrial sampling is an important diagnostic tool in gynecologic practice and is used in the evaluation of abnormal uterine bleeding, for endometrial dating, and in the assessment of endometrial histologic results in women receiving hormonal therapy. Traditionally, curettage has been the gold standard sampling technique, although it is being replaced by simpler, less morbid, and less expensive office biopsy techniques. The Novak (Miltex, Inc., Lake Success, N.Y.) curette and vacuum aspirator have been shown to be as accurate as curettage when endometrial histologic results are compared.2

The Pipelle (Unimar Inc., Wilton, Conn.) endometrial suction curette was developed as a method of endometrial sampling, causing less patient discomfort while obtaining adequate tissue for histologic diagnosis. To date, few comparative trials have been conducted comparing this sampling device with other, proved methods of endometrial sampling.8-9

The purpose of this study is to compare sample adequacy and pain associated with sampling and to correlate endometrial sampling histologic findings, with

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hysterectomy histologic results in specimens obtained by the Pipelle and Novak curettes.

Material and methods

During a 2-year period ending February 1990, women with abnormal uterine bleeding seen at the Gailor Clinic, the ambulatory care facility of the University of Tennessee, Memphis, were enrolled in this prospective trial. Only women with a positive pregnancy test result or not wishing to participate were excluded. Patients were randomized according to computer-generated numbers to undergo endometrial sampling with either the Novak curette10 (group 1) or the Pipelle (group 2) endometrial suction curette.

The Pipelle device is made of a clear, flexible polypropylene sheath. It has a blunt, rounded distal tip and measures 23.5 cm in length. It has inner and outer diameters of 2.6 and 3.1 mm, respectively. By withdrawing on the inner piston, a negative pressure is created within the endometrial cavity. The Novak curette is a stainless steel curette with an outer diameter of 5 mm. Serrated edges surround its distal opening. A 10 ml plastic syringe was used to create a negative

The position of the uterus was determined by pelvic examination, and the biopsy was performed after the cervix was exposed and cleaned with a povidone-iodine solution. No premedication was used for any patient. The Novak curette was inserted into the endometrial

Table I. Comparison of demographic data and sampling between Novak and Pipelle groups

	Novak group (N = 126)	Pipelle group (N = 149)	p·Value
Age (yr)			
Median	44	40	0.0005
Range	21-80	25-66	
Gravidity			
Median	5	4	NS
Range	0-17	0-25	
Parity			
Median	4	4	NS
Range	0-17	0-18	
Biopsy indication			
Premenopausal abnormal uterine bleeding			
No.	105	134	NS
%	83.3	89.9	
Postmenopausal bleeding			
No.	21	15	NS
%	89.9	10.1	

NS, Not significant; $p \ge 0.05$.

cavity and the specimen was obtained from the fundal portion. The Pipelle device was introduced into the endometrial cavity, and the piston was then withdrawn to the full length of the instrument, thereby creating a negative pressure. As the instrument was withdrawn, it was rotated, drawing tissue into the lumen. After the Pipelle device was removed, the tip was cut off and the sample expelled. All biopsy specimens were placed in 10% formaldehyde and sent for tissue processing and staining. The pathologist interpreting the endometrial samples was blinded to the instrument used to obtain the sample.

The patient's age, gravidity, parity, and menstrual history were noted. Outcomes measured included specimen adequacy, i.e., the amount of tissue necessary for the pathologist to make a diagnosis. An adequate sample was defined as one or more pieces of endometrium large enough to determine the gland-to-stroma ratio and endometrial morphologic features. An inadequate sample was defined as consisting only of blood or cervical mucus with fragments of benign endocervix or a large amount of blood with only small fragments of endometrial glands and stroma. In those patients requiring hysterectomy, pathologic findings on endometrial biopsy were compared with endometrial histologic results at the time of hysterectomy.

After the biopsy, the patient was asked to quantify the intensity of any pain experienced during the procedure. Pain was graded on a scale of 0 to 10, with 0 being no pain and 10 being severe pain with cramping.

Statistical analysis was performed with a two-tailed Student t test and two-tailed Fisher's exact test, with a p value of < 0.05 considered significant.

Results

Two hundred seventy-five patients were enrolled in this study, 149 (54.2%) undergoing Pipelle device endometrial sampling and 126 (45.8%) having Novak curette sampling. No uterine perforations or other complications were noted in either group. Table I compares the two groups with respect to patient age, gravidity, and parity. Patients in the Novak group were statistically older (p = 0.005) than those in the Pipelle group. Thirty-six (13.1%) of the patients were postmenopausal. The inadequacy rate in this group of patients was 4 of 18 (22.2%) in the Pipelle group and 5 of 18 (27.8%) in the Novak group. Table I also details the indications for endometrial sampling in the two groups. There were no significant differences between groups.

Patients undergoing Novak endometrial sampling had a mean pain score of 4.36, with 22 of 126 (17%) reporting a pain score of >7 (severe pain) and only 39 of 126 (31%) reporting a score of <3 (minimal pain). Patients undergoing Pipelle endometrial sampling had a mean pain score of 3.21, with 10 of 149 (7%) reporting a pain score of >7 (severe pain), whereas 60 of 149 (40%) reported a pain score of <3 (minimal pain). Patients were further subdivided by menstrual days, gravidity, parity, and menopausal status. Those reporting the least pain were in the Pipelle group during the second menstrual week, whereas those reporting the most pain were characterized by Novak biopsy after the third menstrual week. Although there was a significant difference between the two groups (p < 0.05) with regard to pain induced by biopsy, there was no difference within each group with regard to timing of the biopsy during the menstrual cycle.

The histologic results of the biopsies are outlined in Table II. Although no patient had a failed endometrial biopsy, insufficient tissue for histologic diagnosis was obtained in 19 (12.8%) patients in the Pipelle group compared with 12 (9.5%) in the Novak group (p > 0.05). Patients in whom sufficient tissue for histologic analysis was not obtained subsequently either

Sampling method	Endometrial histologic finding							
	Endometritis	Hyperplasia	Proliferative or secretory	Insufficien tissue				
Pipelle ($N = 149$)								
No.	23	11	96	19				
%	15.4	7.4	64.4	12.8				
Novak $(N = 126)$								
No.	23	15	76	12				
%	18.3	11.9	60.3	9.5				

Table II. Comparison of endometrial histologic results between Novak and Pipelle groups

had repeat endometrial sampling or underwent curettage. No patient in this group was found to have either endometrial hyperplasia or carcinoma.

Fifty patients underwent hysterectomy subsequent to endometrial sampling, 26 (17%) in the Pipelle group and 24 (19%) in the Novak group. In 48 of 50 (96%) patients, the endometrial histologic results at the time of hysterectomy were in agreement with the endometrial sampling histologic results. The hysterectomy specimens of one patient in each group differed significantly from the biopsy findings. In the Novak group a mixed müllerian tumor was found, whereas endometrial hyperplasia without atypicality was found in the Pipelle group. In both patients endometrial sampling had revealed proliferative endometrium.

Comment

This randomized clinical trial suggests that the Pipelle device is as effective as the Novak curette in obtaining endometrial tissue for histologic evaluation. Both the adequacy and quality of the samples obtained by the two methods appeared similar. The study had a 0.129 power of detecting a difference in inadequacy rates of 12.8% and 9.5% between the Pipelle and Novak groups, respectively. To detect a similar difference at a power of 0.80, a sample size of 1507 patients would be required in each group. Thus no definite conclusion can be made in this regard. Unlike other trials,10 this patient population was made up of 13.1% of women who were postmenopausal. The inadequacy rates in postmenopausal patients were 22.2% and 27.8% in the Pipelle and Novak groups, respectively. In this group of older patients, adequate tissue for histologic analysis was obtained in 27 of 36 patients, thereby eliminating the risk and cost of curettage for these patients. Because the Pipelle has a smaller diameter, there is less difficulty in passing the instrument through the cervix and therefore less pain. The smaller size also allows potential entry into uteri with relative stenotic cervices as seen in some menopausal patients. As a result, the Pipelle device may be a preferable instrument in selected postmenopausal patients.

The Pipelle device was associated with less discomfort than was the Novak curette. This was most likely related to its flexibility and smaller caliber. The Pipelle device can be made more rigid by placing it in the freezer, but in this study the Pipelle devices were not pretreated. Although the women in the Novak group were older, confounding is unlikely to have influenced the findings. When the results were reevaluated to eliminate age bias, the results were not significantly altered. In addition, chance also is an unlikely explanation for this difference. This study had a 0.898 power of detecting a difference in pain score as large as 3.21 versus 4.36. Although previous studies revealed the Pipelle to be well tolerated,4-6 to our knowledge this study is the first to quantify patient discomfort. This decreased pain may be a result of the Pipelle's smaller diameter and flexible tip. There also may be an effect of the age differences found between the two groups.

Two patients had an inaccurate diagnosis made at the time of endometrial biopsy. Because not all patients underwent hysterectomy, it is certainly possible that other lesions were not diagnosed. However, it is important to remember that even curettage does not sample the entire endometrium and will, on occasion, not identify a clinically important lesion. The single-use disposable Pipelle device costs \$6 per unit. The initial cost of the nondisposable Novak curette is \$31. Sterilization and packaging and the purchase of the required syringe are additional costs that are incurred with each use for the lifetime of the Novak instrument. However, because the Novak curette's expected useful lifetime is unlimited, whereas a new Pipelle device must be used for each biopsy, the cost factor favors the Novak curette as the number of biopsies performed increases. In fact, depending on sterilization and packaging costs, the Pipelle device may result in increased costs over the Novak curette. The cost of processing the tissue sample and histologic interpretation is the same for both methods.

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Spontaneous preterm birth: A case-control study

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Information on demographic characteristics, life-style, and reproductive, prenatal, and medical history was obtained from the prenatal records of 140 women with a preterm delivery (cases) and 280 women with a term delivery (controls) matched by age. Compared with controls, a greater proportion of cases were nonwhite, never married, and educated <12 years. When logistic regression was used to control for confounding variables, a number of risk factors were found to be associated with an increased risk for preterm delivery: a history of a prior preterm delivery (relative risk, 3.5; confidence interval, 1.6 to 7.8), smoking during the pregnancy (p value test of trends, 0.005), and a prepregnancy weight <61.5 kg (relative risk, 2.0; 95% confidence interval, 1.2 to 3.2). Seven (5%) cases and none of the controls indicated a history of maternal diethylstilbestrol exposure (p < 0.001). A history of prior induced abortion was associated with a modest increase in risk for a spontaneous preterm delivery (relative risk, 1.6; confidence interval, 0.9 to 2.7), and this risk increased with increasing numbers of induced abortions (p value for χ^2 test of trend, 0.02). (AM J OBSTET GYNECOL 1991;165:1290-6.)

Key words: Preterm delivery, case-control study, obstetric history, cigarette smoking, maternal weight

In the United States, preterm delivery, defined as the birth of a fetus before 37 completed weeks of gestation, occurs in about 9% of all single live births and accounts for a large proportion of neonatal morbidity and mortality. Approximately two thirds of all preterm births occur spontaneously after premature labor or premature rupture of the membranes. The remaining preterm births are due to adverse maternal or fetal diagnoses that warrant an early delivery, such as hypertension, antepartum hemorrhage, diabetes, or fetal growth

retardation.³ This report seeks to examine risk factors for spontaneous preterm delivery that could be identified from the prenatal history, and our results are based on a case-control study in which medical records were used.

Methods

Cases were defined as women who were delivered of a live singleton infant between 20 and 37 weeks' gestation and in whom the delivery had been preceded by spontaneous labor or rupture of the membranes without induction for maternal or fetal indications. Cases were selected from all deliveries between Oct. 1, 1988, and March 31, 1989, at Brigham and Women's Hospital. We identified 263 deliveries that started spontaneously before 37 weeks (ICD-9-CM codes 644.2, 658.1, and 658.2). Gestational age at delivery was defined by last menstrual period (LMP) confirmed by clin-

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Table I. Distribution of various neonatal outcomes in cases and controls

	Cases (1	n=140)	Controls $(n = 280)$		
	No.	%	No.	%	
Gestational age (wk)					
≥20-<24	5	3.6			
≥24-<28	5	3.6			
≥28-<32	13	9.3			
≥32-<34	19	13.5			
≥34-<36	55	39.3			
≥36-<37	43	30.7			
≥37 `			280	100.0	
1 min Apgar score				3	
'≤5	22	15.7	14	5.0	
>5	118	84.3	266	95.0	
5 min Apgar score				•	
≤7	20	14.3	5	1.8	
>7	120	85.7	275	98.2	
Condition at discharge	•		· ·		
Alive	130	92.9	280	100.0	
Dead	10	8.1	0	0.0	
Sex					
Male	60	42.9	· 126	45.0	
Female	80	57.1	154	. 55.0	
Congenital abnormalities				•	
Yes '	12	8.6	10	3.6	
No ·	128	91.4	270	96.4	

ical examination or ultrasonography. We excluded 14 cases with inadequate criteria to classify them as <37 weeks. Also excluded were 109 women transferred to Brigham and Women's Hospital from surrounding community hospitals since they lacked prenatal information that was comparable to that for nontransferred women. The remaining 140 cases were available for analysis.

Women selected as controls were delivered after spontaneous labor at >37 weeks' gestation by similar standards. They were matched to cases by delivery date (closest preceding or subsequent delivery) and maternal age within 1 year. Women transferred from surrounding hospitals were not eligible and two controls were matched to each case.

Demographic characteristics, life-style variables, and reproductive, prenatal and medical history were obtained through a medical record review. Smoking history pertained to smoking during the current pregnancy as reported at the first prenatal visit. Analyses regarding the effect of a prior miscarriage, induced abortion, or preterm delivery excluded primigravid subjects. The χ^2 test and Fisher's exact probability test were used to compare case-control differences for discrete variables and the Student t test was used for the analysis of continuous variables. Relative risk estimates and 95% confidence intervals were calculated and adjusted for confounding factors by means of unconditional logistic regression. Because of the matching design, conditional logistic regression was used to verify all unconditional logistic regression estimates, and any substantial variations are reported in the results.

Results

Table I shows the distribution of the cases and controls by neonatal outcome. The cases delivered after a mean gestational age of 236 days (33 weeks 5 days) and the controls after 278 days (39 weeks 5 days). The mean birth weight of babies born to mothers who had a preterm delivery (cases) was 2328 ± 714 gm compared with a mean of 3496 ± 446 gm in babies born to mothers at term (controls). A greater proportion of cases were delivered of babies with 1-minute Apgar scores of ≤ 5 and 5-minute Apgar scores of ≤ 7 . Ten preterm babies died before discharge from the hospital; six of them had a gestational age <26 weeks. None of the babies of the controls died before discharge. The ratio of boys to girls was 1.33 for the cases and 1.22 for the controls (p = 0.72). Among cases, 12 babies had a congenital malformation (8.6%), compared with 10 (3.6%) babies born to control mothers (p = 0.03). The group of babies with congenital malformations had a variety of diagnoses including minor malformations such as polydactyly and major abnormalities such as trisomy 21 syndrome.

Demographic characteristics of the cases and controls are shown in Table II. A greater proportion of cases were nonwhite, single, and had completed <12 years of schooling. These demographic risk factors are included as adjustment variables in analyses relating to personal characteristics reported below.

A slightly greater proportion of controls than cases were primigravid (31.1% vs 27.9%, Table III). There was no increased risk of a preterm delivery with increasing gravidity or parity (Table III). To examine the

Table II. Demographic characteristics of cases and controls

·	Cases			Controls				
i	Total No.*	No.	%	Total No.*	No.	%	Crude relative risk	95% Confidence interval
Age (yr)	140	,		280				
<25		36	25.7		71	25.4		
25-29		34	24.3		74	26.4		
30-34		45	32.1		89	31.8		
≥35		25	17.9		46	16.4		
Race	136			273				
White		75	55.I		181	66.3	1.0	•
Nonwhite		61	44.9		92	33.7	1.6	1.0-2.6
Marital status	140	•		280				
Ever married		104	74.3		236	84.3	1.0	
Never married		36	25.7		44	15.7	1.9	1.1-3.3
Educational level	124	r		268				
(yr)								
≥12		105	84.7		244	91.0	1.0	
<12		19	15.3		24	9.0	1.8	1.0 - 3.5

^{*}Total number varies because of missing information.

Table III. Relative risk estimates for preterm delivery by reproductive history

		ises 140)		Controls $(n = 280)$		tive risk	,
	No.	% :	Ņo.	%	Crude	Adjusted	95% Confidence interval
Gravidity*†							
1	39	27.9	87 .	31.1	1.0	1.0	
2	37	26.4	85	30.3	1.0	, 1.0	0.6-1.8
2 3	26	18.6	47	16.8	1.2	1.4	0.7-2.7
≥4	38	27.1	61	21.8	1.4	1.2	0.6-2.2
Parity*†							
1	71	50.7	138	49.3	1.0	1.0	
2	41	29.3	95	33.9	0.8	0.8	0.5-1.4
≥3	28	20.0	47	16.8	1.2	0.8	0.4-1.5
Miscarriage‡							
0	71	70.3	131	67.9	1.0	1.0	
Any	30	29.7	62	32.1	0.9	1.0	0.5-1.8
1	20	19.8	47	24.3	0.8	0.9	0.5-1.7
≥2	10	- 9.9	15	7.8	1.2	1.4	0.5-3.7
Induced abortion†	,				•		
0	48	47.5	116	60.1	1.0	1.0	
Any	53	52.5	77	39.9	1.7	1.6	0.9-2.7
1	33	32.7	54	28.0	1.5	1.5	0.8-2.7
≥2	20	19.8	23	11.9	2.1	1.9	0.8-4.3
Preterm birth†		-					
0	80	79.2	179	92.7	1.0	1.0	
Any	21	20.8	14	7.3	3.4	3.5	1.6-7.8
1	14	13.9	11	5.7	2.9	2.7	1.0-6.8
≥2	7	6.9	3	1.6	5.2	6.7	1.6-28.1

^{*}Adjusted for race (white, nonwhite), marital status (ever married, never married), educational level (≥12, <12), and age (continuous).

effect of prior pregnancy outcomes, primigravid cases and controls were excluded. The restricted data set showed a similar proportion of miscarriages but a greater occurrence of induced abortions among cases than controls. Women with a history of induced abortions were at a greater risk of a preterm delivery compared with those with no such history, but this was of borderline statistical significance. The relative risk estimates increased with increasing numbers of past induced abortions (*p* value for test of trend, 0.02). A

[†]Gravidity and parity include the index pregnancy for cases and controls.

[‡]Restricted to gravidity ≥ 2 and adjusted for race (white, nonwhite), marital status (ever married, never married), educational level (≥ 12 , <12), gravidity (2, 3, or ≥ 4), and age (continuous).

Table IV. Relative risk estimates for preterm delivery by cigarette smoking during this pregnancy

	Cases (n = 129)		Controls $(n = 270)$		Relative risk			
	No.	%	No.	%	Crude	Adjusted*	95% Confidence interva	
Cigarette smoking†								
No‡	98	76.0	233	86.3	1.0	1.0		
Yes	31	24.0	37	13.7	2.0	1.6	0.9-2.9	
No. of cigarettes per day								
≤5	7	5.4	13	4.8	1.3	1.1	0.4-3.0	
6-10	10	7.8	11	4.1	2.2	1.8	0.7-4.7	
>10	14	10.8	13	4.8	2.6	2.0	0.8-4.9	

^{*}Adjusted for race (white, nonwhite), marital status (ever married, never married), and educational level (≥12, <12).

Table V. Relative risk estimates for preterm delivery by height and prepregnancy weight

	Cases			Controls					
	Total No.*	No.	%	Total No.*	No.	%	Relative risk†	95% Confidence interval	
Height (m)	135			267					
≥1.60		78	57.8		163	61.0	1.0		
<1.60		57	42.2		104	39.0	1.1	0.7-1.6	
Prepregnancy weight (kg)	117			241					
≥61.5		42	35.9		124	51.5	1.0		
<61.5		75	64.1		117	48.5	2.0‡	1.2-3.2	
Quetelet's index§	114			232					
≥24.5		33	29.0		83	35.8	1.0		
>22.0, <24.5		29	25.4		75	32.3	1.2‡	0.6-2.2	
≤22.0		52	45.6		74	31.9	2.0‡	1.1-3.6	

^{*}Total number varies because of missing information.

history of a preterm birth was reported in 21 (20.8%) of the multiparous cases compared with 14 (7.3%) of the multiparous controls (p < 0.01). This indicates a strong association between a prior preterm birth and prematurity. Compared with women with no history of preterm deliveries, women with one prior preterm delivery and women with two or more prior preterm deliveries had a 2.7-fold and 6.7-fold risk of subsequent preterm delivery, respectively.

Table IV indicates that cigarette smoking during the index pregnancy was associated with a relative risk for preterm delivery of 1.6 (95% confidence interval, 0.9 to 2.9). The relative risk estimates of a preterm delivery increased with increasing number of cigarettes smoked per day (p value for test of trend, 0.005) with the greatest risk in women who smoked >10 cigarettes per day during pregnancy.

Cases and controls were similar with respect to height, whereas the mean prepregnancy weight was substantially less in cases than in controls (Table V). This persisted after additional adjustment for smoking (relative risk, 2.0; 95% confidence interval, 1.2 to 3.2). When a ratio of weight to height (Quetelet's index) was calculated and stratified by mean tertiles within the control group, we observed an increase in risk with decreasing weight-to-height ratios (p value for test of trends, 0.06).

Table VI shows the two-way interaction of maternal weight and cigarette smoking. Compared with nonsmokers who were ≥61.5 kg, nonsmokers who weighed less had a 2.1-fold increase in risk. Smokers in the same weight categories also showed a twofold increase in risk. The greatest risk for a preterm delivery was observed in underweight women who also smoked.

Table VII shows the distribution of a number of medical and gynecologic conditions among the cases and controls. Our case group of spontaneous preterm deliveries excluded cases in which labor was induced because of medical conditions; therefore no significant difference in the frequency of diabetes, asthma, chronic hypertension; and mitral valve prolapse was observed in this study. No significant differences were noted in

[†]Excludes 11 cases and 10 controls with missing information.

[‡]Reference group.

[†]Adjusted for race (white, nonwhite), marital status (ever married, never married), and educational level (≥12, <12).

[‡]Additional adjustment for smoking.

[§]Quetelet's index: Weight (in kilograms)/height in meters2.

Table VI. Relative risk estimates for preterm delivery by prepregnancy weight and cigarette smoking

			ses 111)		trols 233)		· .
Weight (kg)	Smoking :	No	%	No.	%	Relative risk*	95% Confidence interval
≥61.5 ≥61.5 <61.5 <61.5	No Yes No Yes	30 11 53 17	27.0 9.9 47.8 15.3	103 16 98 16	44.2 6.9 42.0 6.9	1.0 2.2 2.1 3.1	0.9-5.7 1.2-3.7 1.3-7.4

^{*}Adjusted for race (white, nonwhite), marital status (ever married, never married), and educational level (≥12, <12).

Table VII. Distribution of medical and gynecologic conditions among cases and controls

	Cases			Controls			
	Total No.*	No.	%	Total No.*	No.	%	p Value
Medical condition		`,		. ,	, .		
Diabetes mellitus	140	4	2.9	. 280	3	1.1	0.23
Asthma		9	6.4		10	3.5	0.18
Chronic hypertension	•	2	1.4		. 6	2.1	0.72
Mitral valve prolapse		5	3.6		12	4.3	0.73
Gynecologic condition	•		•				
History of subfertility†	104	8	7.7	236	13	5.5	0.44
Papanicolaou smear‡	93			214	•	, ,	ē
Dysplasia		4' '	4.3	•	6	2.8	0.50
Atypical changes		. 4	4.3		6	2.8	0.50
Infectious features		42	45.2		77	36.0	0.13
Diethylstilbestrol	140	· . 7	5.0	280	0	0.0	0.0004

^{*}Total number varies because of missing information.

the occurrence of abnormal Papanicolaou smears. However, 7 (5%) of the cases indicated a history of diethylstilbestrol exposure compared with none of the controls (p = 0.0004).

Comment

Several studies have described risk factors associated with a preterm delivery. ⁴⁻⁶ The interpretation of these studies is complicated by the fact that some studies did not distinguish between spontaneous preterm births and induced preterm births and by the fact that definitions of prematurity may vary. In the past the definition of prematurity was based solely on a birth weight <2500 gm or was in conjunction with a gestational age of <37 weeks. In 1980, the World Health Organization recommended that a preterm infant be defined as one delivered at <37 weeks' gestation, regardless of weight.⁷ In spite of these limitations, a comparison of the findings from previous studies and our study is instructive.

A number of demographic variables including age, race, and socioeconomic status have been identified as risk factors for prematurity. Two studies have identified an inverse relationship between the age of the

woman and risk of spontaneous preterm birth.8,9 We chose to control for age by matching cases and controls within I year and hence cannot comment on age as a risk factor per se. We found that nonwhite women had an approximately 60% greater likelihood of delivering a premature infant compared with white women. This is in agreement with the study by Henderson and Kay¹⁰ that assessed gestation and birth weight in a group of black women and a group of white women who were similar in socioeconomic status, educational level, age, smoking, amount of prenatal care, and sex of the fetus. They observed that black infants had a significantly shorter gestation and lower birth weight than white infants. Lower socioeconomic status, as defined by a number of indices, also increases the risk of spontaneous preterm delivery.8, 9 In our study unmarried women who had not completed high school had a greater risk for a spontaneous preterm delivery compared with married women who were high school graduates. Clearly, these demographic risk factors must be taken into consideration when other, more easily modifiable risk factors are examined.

We found a modest increase in risk for spontaneous

[†]Restricted to ever-married women.

[‡]The results from the Papanicolaou smear taken at the first prenatal visit were divided into three groups: (1) presence of dysplasia, (2) atypical changes (atypical benign or atypical squamous), and (3) infectious features (inflammation, polymorphonuclear leukocytes, trichomonas, clue cells, or koilocytosis).

preterm delivery associated with a prior induced abortion that was of borderline statistical significance after controlling for demographic factors and gravidity. The effect of induced abortions has been examined in several studies.11,12 As reported by Levin et al.,13 a greater risk for spontaneous preterm delivery was found after two or more induced abortions. Berkowitz9 also found an increased risk for spontaneous preterm deliveries associated with induced abortion, particularly for second-trimester abortions. Our results confirm the observation by Levin et al. and suggest that women with two or more induced abortions have a twofold risk for preterm delivery. Further study of the effect of induced abortion on risk of preterm delivery is warranted and should focus on additional detail with respect to gestational age and the nature of the procedure (i.e., gestational age at time of procedure and details such as whether laminaria pretreatment was used).

In our study a similar proportion of cases and controls experienced one or more prior spontaneous abortions, defined as a pregnancy loss before 20 weeks of gestation. Earlier studies reported no association with preterm delivery and one or more prior spontaneous abortions during the first trimester. 14, 15 However, the prevalence of a preterm delivery increased substantially in women with a history of one or more spontaneous abortions during the second trimester. 14, 15 We were not able to distinguish first- from second-trimester miscarriages in our data.

The strongest risk factors from the obstetric history that we observed was a history of prior preterm birth noted in 21% of cases and only 7% of controls. This translated into a 3.5 fold-increase in risk for prematurity, which agrees well with the observation of Carr-Hill and Hall, 16 who reported a relative risk of 3.2 for a preterm delivery after one previous preterm delivery and a relative risk of 6.8 when the last two gravidities ended in a preterm birth. In a Norwegian study that included nearly half a million singleton births, similar risks were observed.17

Simpson¹⁸ was the first to report a reduction in birth weight as a result of maternal smoking, and this finding has been confirmed. 19, 20 The risk of a preterm delivery or a low-birth-weight infant increases with greater tobacco use.8.21,22 In our study we found a 60% excess risk for preterm delivery associated with maternal cigarette smoking, and the risk of a preterm delivery rose with increased cigarette smoking per day. Biologic explanations for this association include the nicotine content in tobacco smoke, which can cause constriction of the placental arterial vessels, and the carbon monoxide content in smoke, which can produce hypoxemia of the fetus. Whereas there appears to be substantial epidemiologic and biologic data to support the association between smoking and prematurity, we cannot rule out the possibility that the association of cigarette smoking is confounded by other maternal characteristics. In particular, alcohol consumption, associated with preterm delivery in one study,22 was not examined in this data set because of the absence of consistent information on this variable in the medical records we reviewed.

Our finding of an independent association of preterm delivery with decreasing weight and cigarette smoking confirms the findings of Fedrick and Anderson,8 who reported a decline in the prevalence of preterm deliveries with both increasing weight and decreasing cigarette smoking. An earlier investigator suggested that dietary insufficiencies may be responsible for this association.23 This is a hypothesis clearly in need of further study.

Other gynecologic factors investigated in this study included Papanicolaou smear abnormalities and diethylstilbestrol exposure. Findings such as dysplasia or atypia on the Papanicolaou test done prenatally did not appear to influence risk, although 45% of the cases for whom cytologic results were available showed features of inflammation such as white blood cells, trichomonas, or clue cells, compared with 36% of controls. Further study of cervical infection is warranted because of the growing evidence that vaginal flora plays a role in the pathogenesis of preterm labor.24

Exposure to diethylstilbestrol in utero was identified only in the cases. We found no previous study that linked diethylstilbestrol exposure with spontaneous prematurity, but it is known that diethylstilbestrol exposure has been linked to preterm premature rupture of the membranes.25 We considered the possibility of a recall bias to explain this association because women with preterm birth experience may be motivated to search for likely explanations. However, it is unlikely that this association is explained by the presence of recall bias because only one of the seven women with prior diethylstilbestrol exposure had a history of a preterm delivery. Thus diethylstilbestrol exposure may be a strong, albeit relatively rare, risk factor for spontaneous prematurity.

We believe our findings may have several implications for the prevention of preterm birth. We have confirmed that the demographic profile of the woman who is delivered prematurely includes being nonwhite, unmarried, and less well educated. This provides a target population that is in need of better prenatal care. Smoking is a modifiable risk factor for prematurity that should be more aggressively approached through prenatal counseling and smoking cessation programs. Ascertainment of the obstetric history is extremely important since a prior preterm delivery increases threefold the risk for a future preterm birth. A prior preterm delivery or diethylstilbestrol exposure should prompt a search for predisposing factors such as incompetent cervix. Finally, induced abortion was identified as a possible risk factor, which emphasizes the importance of contraceptive counseling.

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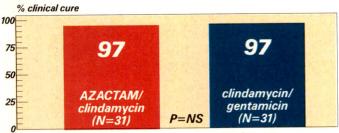
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N POSTPARTUM ENDOMETRITIS*

AZACTAM delivers more than you're expecting...

- Broad aerobic gram-negative in vitro coverage, including <u>Escherichia</u> <u>coli</u>, <u>Proteus</u> <u>mirabilis</u>, and Enterobacter <u>cloacae</u>.[†]
- Greater than 90% cure rates in combination with clindamycin.¹⁻³
- No time-consuming, costly, routine therapeutic drug monitoring.
- May be used in penicillin-allergic patients.[‡]
- Pregnancy Category B.

There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.

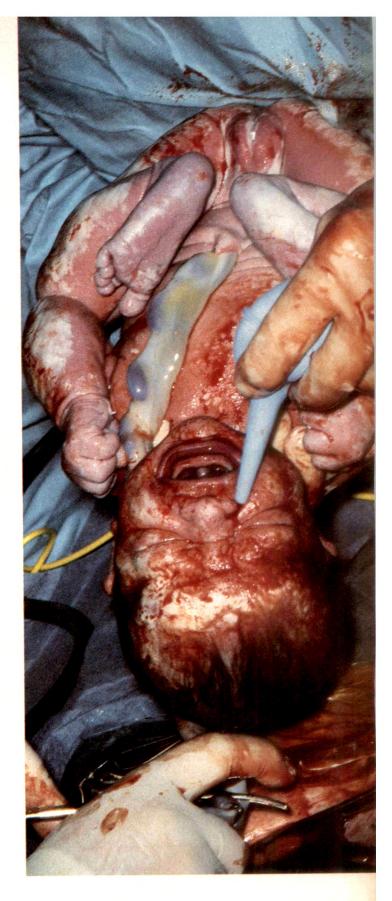


A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.

No universal criteria exist that may satisfy a single definition of "cure." Use of this term may vary between clinical settings and investigators, and the reader is urged to consult the references listed.

Azactam[®] aztreonam IV/IM 1g/2g

A logical alternative to aminoglycosides.



*Due to susceptible organisms.

tAlthough a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

*Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. See full prescribing information for recommendations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions occurred in less than 2% of patients, including local reactions, diarrhea, nausealvomiting, and rash.

Please see adjacent page for brief summary of prescribing information.

Azactam[®] aztreonam IV/IM 1g/2g

AZACTAM® FOR INJECTION Aztreonam For Injection

DESCRIPTION-AZACTAM (Aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowishwhite lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE—Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gramnegative aerobic pathogens seen in general surgery.

Concurrent Therapy— Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organism(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an anti-anaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase in vitro in some gram-negative aerobes such as Enterobacter and Pseudomonas species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These in vitro findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS—Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS-Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to lifethreatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents

present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by Clostridium difficile is one primary cause of "antibiotic-associated colitis."

difficile is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against C. difficile (e.g., vancomycin).

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS—General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility—Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy-Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers—Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use-Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS—Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity: Hypersensitivity—anaphylaxis, angioedema, bronchospasm. Hematologic—pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. Gastrointestinal—abdominal cramps; rare cases of C. difficile—associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). Dermatologic—purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. Cardiovascular—hypotension, transient ECG changes (ventricular bigeminy and PVC). Respiratory—one patient experienced flushing, chest pain, and dyspnea. Hepatobiliary—hepatitis, jaundice. Nervous System—seizure, confusion, vertigo, paresthesia, insomnia, dizziness. Musculoskeletal—muscular aches. Special Senses—tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. Other—vaginal candidiasis, vaginitis, breast tenderness. Body as a Whole—weakness, headache, fever, malaise.

Adverse Laboratory Changes—Those reported without regard to drug relationship during clinical trials were: <code>Hepatic-elevations</code> of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). <code>Hemic-increases</code> in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. <code>Renal-increases</code> in serum creatinine.

OVERDOSAGE—If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION-Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg**, or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231E)

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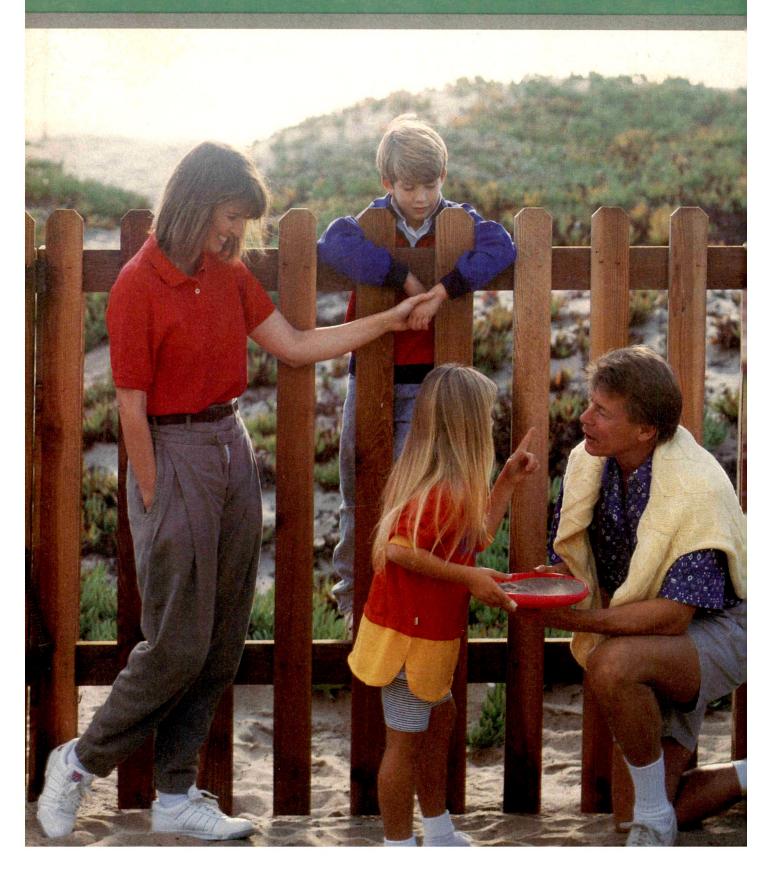
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DRUG ABUSE IS CHILD ABUSE



NORPLANT SYSTEM levonorgestrel implants



feel comfortable recommending it to your patients

Effectiveness comparable to tubal ligation

Although no contraceptive is 100% effective, the NORPLANT SYSTEM is one of the most effective forms of birth control ever developed. The average annual gross pregnancy rate over a five-year period is less than 1%.

Lowest expected and typical failure rates with NORPLANT SYSTEM vs. sterilization during first-year use



Adapted from Trussell J, et al.

Long-term convenience... estrogen-free

NORPLANT SYSTEM, a progestin-only contraceptive, is an excellent option for women desiring a long-term method. Its effectiveness lasts for five years and is not dependent on compliance.

Reversible, with prompt return to previous level of fertility

NORPLANT SYSTEM is reversible at any time; the contraceptive effect ceases when the capsules are removed.

In one study, 40% of women wanting to conceive did so within 3 months of removal, 63% by 6 months, 76% by 12 months, and 90% by 24 months.²

High patient acceptance despite menstrual pattern changes

NORPLANT SYSTEM causes menstrual irregularities (the most common complaint), which occur in many women during first-year use and diminish over time. Only 9.1% of patients discontinued during first-year use due to these changes.^{3†}

Women who are thoroughly informed of potential menstrual changes and side effects are more tolerant of them when they occur.⁴

Well-received, well-liked by American women

One study of 140 users revealed that 94% were satisfied with NORPLANT SYSTEM, 91% recommended it to their friends, and 74% wanted to use it again.⁵

A 10- to 15-minute office procedure

A local anesthetic and a small 2 mm incision...little or no discomfort during insertion...proper placement should not leave a noticeable scar...in most cases sutures are not needed.

*Serious as well as minor side effects may occur (see prescribing information).

† Based on multicenter trials of 2,470 women.

Please see brief summary of prescribing information on adjacent page.



Lasts 5 years...yet is reversible

Table II. Five other cases that showed dissociation between serum levels of CA 125 and CA 130

Case No.	Age (yr)	CA 125	CA 130	· Clinical course
1*	42	106	. 14	Adenomyosis
		, 129	24	(after hysterectomy)
2†	43	270	11	NED, 4 yr
3†	55	. 216	9	NED, 4 yr
4†	39	2236	. 6	NED, 5 yr
5†	69	5461	14	NED, 5 yr

NED, No evidence of disease.

except for a small leiomyoma. Interestingly, after removal of the uterus and both ovaries the postoperative serum CA 125 levels increased from the preoperative levels. CA 130 levels measured simultaneously in the sera were consistently low. Therefore a discrepancy between CA 125 and CA 130 levels was observed. The analysis of CA 125 and CA 130 epitopes suggested that the molecule possessing at least two epitopes of CA 125 existed in the sera of this patient, but this molecule lacked the epitopes of CA 130. However, the results of immunohistochemical staining for both CA 125 and CA 130 in the ovarian surface epithelium were positive, implying that both CA 125 and CA 130 existed in the tissues of this patient. Consequently, either the release: mechanism of these epitopes into the serum or the metabolism of these epitopes in the serum may differ in this patient.

Although the exact mechanism of this discrepancy between the serum levels of CA 125 and CA 130 is unclear, this case suggests that healthy women may

rarely have extraordinarily high serum levels of CA 125. Retrospective analysis of CA 125 and CA 130 levels in >2000 women in gynecologic clinics found five patients with high CA 125 levels but low CA 130 levels (Table II). Clinical examinations found no apparent CA 125-producing disorders in those five patients. We have never examined a patient with high CA 130 levels and low CA 125 levels. This implies that CA 130 levels, rather than CA 125 levels, seem to correlate well with pathologic changes in the patients.

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^{*}Laparotomy was performed only in case 1.

[†]Clinical examination did not reveal abnormalities and laparotomy was not performed.



BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION. Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Active thrombophlebitis or thromboembolic disorders.
 Undiagnosed abnormal genital bleeding.
 Known or suspected pregnancy.
 Acute liver disease; benign or malignant liver tumors.
 Known or suspected carcinoma of the breast.

- A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

 1. Bleeding Irregularities Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, and amenormee may occur, and could mask symptoms of cervical or endometral cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.
- 2. Delayed Follicular Atresia Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be
- 3. Ectopic Pregnancies Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out extends pregnancy in an extent of pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent pregnancy in any extent present prognancy in any extent pregnancy in any extent pregn
- ectopic pregnancy in any patient presenting with lower-abdominal pain.

 4. Breast-feeding Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after
- Solution.

 5. Foreign-body Carcinogenesis Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.
- OF International Contractors Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

 B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

- Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

 1. Cigarette Smoking Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

 2. Elevated Blood Pressure — Increase in blood pressure has been reported in combination OC
- users; prevalence increases with long exposure.

 3. Thromboembolic Disorders and Other Vascular Problems An increased risk of
- 3. Intromobemobilic Disorders and Other Vascular Problems An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.
 Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hyperatoric hyperatoric handless.

hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is

In thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of

levonorgestrel-containing OCs.

4. Carcinoma — Recent evidence in the literature suggests no association between OC use and

- increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms. 5. Hepatic Tumors — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal
- hemorrhage.

 6. Ocular Lesions Retinal thrombosis is associated with OC use and is believed to be related to 6. Ocular Lesions — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if their sunexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately. 7. Use Before or During Early Pregnancy — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. Galibladder Disease — Early studies reported an increased lifetime relative risk of galibladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

 Physical Examination and Follow-up — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should

bleeding. Women with a strong raminy instory of breast cancer or who have breast flouries should be monitored with particular care.

2. Carbohydrate Metabolism — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See Warning)

Warnings.) 3. Liver Function -- Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. Fluid Retention — Steroid contraceptives may cause some degree of fluid retention. Prescribe

- with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.
- 5. Emotional Disorders Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

 6. Contact Lenses Contact-lens wearers who develop visual changes or changes in lens
- tolerance should be assessed by an ophthalmologist.

 7. Insertion and Removal Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If a capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. See Full Prescribing Information for
- Detailed Insertion/Removal Instructions.

 8. Infections Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.
- 9. Expulsion Expulsion of capsules was uncommon; frequency increased when capsule 9. Expulsion — expulsion or capsules was uncommon; requency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

 10. Provisions for Removal — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of the page of pages.
- of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and trilodothyronine uptake

CARCINOGENESIS: See Warnings section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See Warnings section and Full Prescribing Information.

NURSING MOTHERS: See Warnings section and Full Prescribing Information.

NIFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal. Adverse Reactions

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site – usually transient – (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects – based on 849 removals – (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis, and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Desage and Administration
The NORPLANT SYSTEM consists of six Silastic* capsules, each containing 36 mg of the progestin, levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. (See Full Prescribing Information for Detailed Insertion/Removal Instructions.)

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Severe obstructive sleep apnea and associated snoring documented during external tocography

David M. Sherer, MD, Christine B. Caverly, RN, and Jacques S. Abramowicz, MD Rochester, New York

Severe obstructive sleep apnea, a disturbance of sleep resulting from intermittent periodic obstruction of the upper airway, was diagnosed after cardiotocography during which the external tocodynamometer disclosed a pattern initially thought to represent uterine activity. In spite of this obstructive sleep apnea with associated markedly low, hypoxic oxygen saturation levels and daytime somnolence of 10 to 12 hours from 32 weeks until delivery, subsequent fetal outcome was good. (AM J OBSTET GYNECOL 1991;165: 1300-1.)

Key words: Pregnancy, obstructive sleep apnea, cardiotocography

Cardiotocography is a widely accepted mode of monitoring both the fetal heart rate and maternal uterine activity. Artifacts of both components are well documented and include doubling and halving of the fetal heart rate and increased fetal heart rate variability with Doppler ultrasonography systems. The external tocodynamometer, a nonquantitative measure of the duration and amplitude of uterine contractions, may nonintentionally pick up fetal activity, maternal coughing, maternal respirations, or changes in maternal position. We describe an unusual case in which intermittent periods of apnea and labored breathing with associated snoring recorded by external tocography subsequently led to the diagnosis of severe obstructive sleep apnea in pregnancy.

Case report

A 27-year-old obese, white patient with insulin-dependent diabetes mellitus since age 14 was followed up during her first gestation at Strong Memorial Hospital. Ocular cataracts were her only diabetic complication. She was first seen at 12½ weeks' gestation, at which time she weighed 207 pounds. Gestational dating was confirmed by ultrasonography. Glycosylated hemoglobin was 8.4%. Maternal serum α -fetoprotein was elevated at 3.30 multiples of the median, after correction for insulin-dependent diabetes. Ultrasonographic evaluation, including a detailed echocardiogram, disclosed normal fetal anatomy. Amniocentesis revealed an elevated amniotic fluid α -fetoprotein level of 2.6 multiples of the median. Acetylcholine esterase electrophoresis

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Received for publication April 25, 1991; accepted May 15, 1991. Reprint requests: David M. Sherer, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, University of Rochester School of Medicine and Dentistry, Strong Memorial Hospital, 601 Elmwood Ave., Box 668, Rochester, NY 14642-8668. was negative. A balanced chromosomal translocation was identified in all 20 cells analyzed, the fetal karyotype being 46, XY, t(2;14) (p23;q32). Maternal karyotype was normal; paternal karyotype was not obtainable. The blood glucose level was maintained between 80 and 150 mg/dl. Weekly nonstress tests were initiated at 32 weeks' gestation and were reactive. Approximately at this time the patient began complaining of excessive daytime somnolence. Beginnning at 35 weeks' gestation, the patient fell asleep during every nonstress test, and frequent, recurrent apneic episodes of 15 to 20 seconds' duration, followed by labored breathing and loud snoring, were noted. This respiratory pattern was recorded on the external tocodynamometer with the transducer positioned as usual over the maternal abdomen (Fig. 1). By 36 weeks, the patient was sleeping between 10 and 12 hours throughout the daytime and falling asleep in inappropriate situations—during conversations with friends and while smoking cigarettes. Further investigation of the sleep disorder was not possible at this time because of the patient's noncompliance.

At 37 weeks, mild preeclampsia ensued with blood pressure of 140/90 mm Hg, pedal edema (+3), and proteinuria (+3). Ear, nose, and throat examination disclosed a large tongue base, a relatively small pharyngeal arch with a large uvula, hypertrophy of the inferior nasal turbinates, and redundant nasopharyngeal mucosa, associated with obstructive sleep apnea. Ultrasonography demonstrated a vertex-presenting fetus with an estimated fetal weight of 2665 gm and decreased amniotic fluid volume. With an amniocentesis revealing a lecithin-sphingomyelin ratio of 3.3 and positive phosphatidylglycerol, delivery was elected. After failed efforts at intravenous oxytocin induction of labor, during which the patient slept almost continuously with the obstructive apnea pattern, cesarean section was performed while she was under epidural anesthesia. A male infant weighing 2780 gm was delivered with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. Umbilical cord arterial pH was 7.28, Po2 was 27 mm

An extraordinarily high CA 125 level in a woman without apparent pathologic foci of CA 125 production: Dissociation between serum levels of CA 125 and CA 130

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We describe a patient with high serum CA 125 levels before and after surgery (>403 U/ml) who had no apparent pathologic foci of CA 125 production. Serum levels of CA 130, which exists on the same glycoprotein as CA 125, were within normal ranges (<35 U/ml) before and after surgery. (AM J OBSTET GYNECOL 1991;165:1297-9.)

Key words: CA 125, CA 130

Recent monoclonal antibodies 130-22 and 145-9 have been reported to recognize the epitope of CA 130, which exists on the same glycoprotein as CA 125.1.2 CA 130 levels correlate well with CA 125 levels in both sera and various tissues, and the coefficient correlation is 0.98.1.2 Moreover, the staining for CA 130 correlates well immunohistochemically with that of CA 125. However, we recently encountered a woman with extraordinarily high serum CA 125 levels who had no apparent pathologic foci of CA 125 production and whose serum CA 130 levels were always within normal ranges.

Case report

A 51-year-old gravida 4, para 2 menopausal woman participating in a whole-body-check program was found to have a high CA 125 level (403 U/ml; normal level <30 U/ml). The whole-body check included a pelvic and abdominal computed tomography scan and demonstrated no other abnormalities. The patient had no history of gynecologic diseases. Magnetic resonance imaging revealed a myomatous nodule (3 cm in diameter) in the myometrium, with no other abnormalities in the pelvis. Vaginal cytologic test, hysteroscopy, chest x-ray examination, peripheral blood analysis, serum chemistry studies, and other tumor markers (carcinoembryonic antigen, CA 19-9, and α-fetoprotein) were within normal ranges. The CA 125 level, measured with a CA 125 radioimmunoassay kit (Centocor Inc., Malvern, Pa.) was 805 U/ml. In contrast, the CA 130 level, measured in the same serum sample with a

U/ml o CA125 CA130 2000 1500 1036 1000 500 403 50 40 30-20 10 90 IX X XI IIX I II III IV V VI VII VII IX X

Fig. 1. Serum CA 125 and CA 130 levels (U/ml) before and after operation.

CA 130 radioimmunoassay kit (a double-determinant Received December 18, 1990; revised April 16, 1991; accepted May assay with antibodies 130-22 and 145-9, Daiichi Radioisotope, Tokyo), was 12 U/ml (normal level $\leq 30 \text{ U/ml}$).

A laparotomy revealed no abnormalities in the abdominal cavity except for the nodule in the myome-

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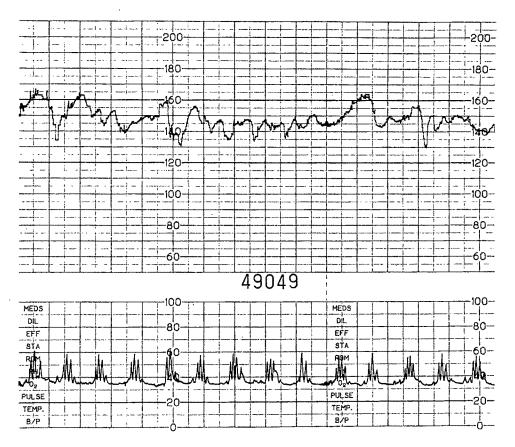


Fig. 1. Cardiotocography at 37 weeks 3 days' gestation. Note frequent regular "uterine activity" subsequently recognized as severe obstructive apnea. Spikes represent labored respiratory movements with snoring. Intermittent straight baseline represents recurrent apneic episodes of 15 to 20 seconds' duration. (Paper speed 3 cm/min.)

Hg, and PCO₂ was 68 mm Hg. The infant's blood glucose level at delivery was 80 mg/dl.

After delivery, an overnight "polysomnogram" (sleep study) showed severe obstructive sleep apnea with oxygen saturation during apnea that generally fell into the low 70% range but occasionally was as low as 63%. The respiratory disturbance index (number of apnea episodes per hour of sleep) was markedly elevated at 144 (normal range, 0 to 5). Treatment with nasal continuous positive airway pressure delivered by nasal mask was not tolerated well by the patient yet elevated the oxygen saturation to 95%.

Comment

The development of severe obstructive sleep apnea in pregnancy, with loud snoring and excessive daytime somnolence, similar to that of our patient has been reported, suggesting that sleep apnea may be either precipitated or exacerbated during pregnancy. It is of interest to note that both these patients had preeclampsia and narrowed oropharyngeal airways with large posterior tongues and redundant pharyngeal mucosa, all contributing factors of upper airway obstruction associated with obstructive sleep apnea. Although both of the reported cases had normal fetal outcomes at

delivery, fetal heart rate decelerations and fetal scalp blood acidosis have been documented during sleep apnea.¹ The long-term effect of recurrent, frequent apneic events on the fetus is unknown. Treatment of this disorder is by either surgical correction of the obstruction or nasal continuous positive pressure delivered by nasal mask.¹

Snoring is known to be associated with a contraction of the abdominal muscles during a substantial part of expiration and an abrupt relaxation of abdominal muscles at the onset of inspiration.² Thus the external tocodynamometer recorded abdominal wall excursion during the snoring with the apneic episode represented by the flat baseline and the labored breathing associated with snoring represented by the elevated baseline and spikes; this is yet another artifact detected by external tocography.

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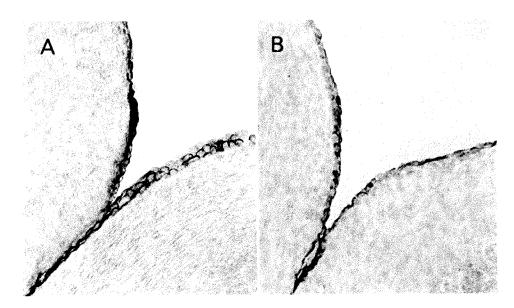


Fig. 2. Results of immunohistochemical staining for CA 125 (**A**) and CA 130 (**B**) were positive in the ovarian surface epithelium. (Original magnification ×400.)

Table I. Double-determinant assays with ¹²⁵I-labeled OC 125 or ¹²⁵I-labeled 130-22 and OC 125- or 145-9-coated beads

	128I-labeled monoclos	nal antibody OC 125	¹²⁵ I-labeled monoclonal antibody 130-22		
Samples	Immunoadsorbent OC 125	Immunoadsorbent 145-9	Immunoadsorbent OC 125	Immunoadsorbent 145-9	
Healthy control	14	< 7.5	<7.5	< 7.5	
Ovarian cancer	392	>500	>500	456	
Our patient	>500	16	12	16	

Results expressed as units per milliliter. Serum levels measured with CA 125 and CA 130 standard antigen from CA 125 radioimmunoassay kits (Centocor Inc., Malvern, Pa.) and CA 130 kits (Daiichi Radioisotope, Tokyo).

trium. A simple hysterectomy with bilateral salpingooophorectomy was performed. The tumor in the myometrium was histologically confirmed to be a leiomyoma.

Fig. 1 shows the CA 125 and CA 130 levels in the sera of the patient before and after surgery. Serum CA 125 levels were consistently high, and the CA 130 levels were consistently within normal ranges. The patient continues to be well with high serum CA 125 levels after the operation.

To analyze the dissociation between serum CA 125 and CA 130 levels in this patient, we developed four systems of a double-determinant assay using monoclonal antibodies OC 125, 130-22, and 145-9. The sera of a patient with ovarian cancer who had high CA 125 levels were used as positive controls. In the case of ovarian cancer, when the CA 125 level was measured by using iodine 125-labeled OC 125 with 145-9-coated beads, the level became higher than the level measured by using ¹²⁵I-labeled OC 125 with OC 125-coated beads (Table I). Similarly, when the CA 130 level was measured by using ¹²⁵I-labeled 130-22 with OC 125-coated

beads, the level became higher than the level measured by using ¹²⁵I-labeled 130-22 with 145-9—coated beads. However, in the serum of our patient, the CA 125 level measured by using ¹²⁵I-labeled OC 125 with OC 125—coated beads was >500 U/ml, but it was 16 U/ml when measured by using ¹²⁵I-labeled OC 125 with 145-9—coated beads. Moreover, CA 130 levels measured by using ¹²⁵I-labeled 130-22 with OC 125— or 145-9—coated beads were <16 U/ml (Table I).

Examination of frozen sections containing ovarian surface epithelium and leiomyoma tissues revealed positive results of tests for both CA 125 and CA 130 (Fig. 2). However, other constitutive elements of the ovary and leiomyoma showed no staining for CA 125 or CA 130.

Comment

A case of extraordinarily high serum levels of CA 125 without apparent pathologic foci of CA 125 production is presented. An exploratory laparotomy revealed no pathologic changes in the abdominal cavity

Management of fetal hemolytic disease by cordocentesis

II. Outcome of treatment

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Forty-eight of 128 pregnancies complicated by maternal red blood cell alloimmunization (49%) received a total of 142 intravascular transfusions (range, 1 to 7) for treatment of severe anemia (hematocrit, ≤30%). Thirteen fetuses (27%) had hydrops when therapy was initiated. The overall survival rate was 96%. Eighty-five percent of survivors received two or more transfusions before delivery. The mean gestational age at initiation of therapy was 28 weeks (range, 18 to 36 weeks). Bleeding from uterine and umbilical cord puncture sites was not of clinical significance. The most common complication was fetal bradycardia (8%). Simple intravascular transfuson resulted in the replacement of fetal red blood cells with adult red blood cells and suppression of fetal erythropoiesis. By the completion of the second transfusion, on average, <1% of circulating red blood cells were fetal. Within 3 weeks of the second transfusion, the mean reticulocyte count was <1%. The rate at which the fetal hematocrit declined after a transfusion (exclusive of the first) was inversely related to gestational age (r = -0.84, p < 0.0001), permitting a 4- to 5-week interval between transfusions after 32 weeks' gestation. A total of 78% of surviving neonates were delivered at term. Neonates transfused more than once antenatally required less phototherapy (75.8 \pm 54 vs 165 \pm 101 hours, p < 0.003) and, when delivered at term, fewer hospital days (4.8 \pm 2 vs 8.6 \pm 6 days, p = 0.01) compared with those transfused once. We conclude that the treatment of fetal anemia by intrauterine simple intravascular transfusion permits a term delivery in the majority of cases and is associated with high perinatal survival and low perinatal morbidity. (AM J OBSTET GYNECOL 1991;165: 1302-7.)

Key words: Fetal hemolytic disease, cordocentesis, fetal intravascular transfusion, immune hydrops fetalis, neonatal hyperbilirubinemia

The first wide-scale fetal therapy was intraperitoneal transfusion for the treatment of fetal hemolytic disease, the success of which varied from center to center. Fetal intravascular transfusion replaced intraperitoneal transfusion as the method of choice for the treatment of fetal hemolytic anemia at the University of Iowa in 1985. We report our experience with the treatment of 48 severely anemic (hematocrit, ≤30%) fetuses who were identified using cordocentesis. The findings demonstrate that simple intravascular transfusion to maintain the fetal hematocrit consistently in a near-normal range (1) results in sustained suppression of fetal cryth-

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ropoiesis, (2) is safe enough to routinely allow a term delivery, and (3) is, as a result of the preceding, associated with low perinatal morbidity and mortality.

Methods

Fetuses of alloimmunized pregnant women and in need of transfusion were identified exclusively with cordocentesis, as previously described.1 Transfusion therapy was initiated when the fetal hematocrit was <30%, because this value is well below the 2.5th percentile for the normal fetus ≥20 weeks. Transfusions were performed as a simple infusion of packed red blood cells (70% hematocrit) into the umbilical vein, as previously described.2 Umbilical venous pressure, pH, Pco2, and Po2 were measured and bicarbonate and base deficit were calculated by standard nomograms. The infusion of blood was halted if the pressure rose significantly above normal.3 Because of the duration of the procedure (20 to 30 minutes) and the volume infused (up to 200 ml), each fetus received pancuronium (0.3 mg/kg estimated fetal weight intravenously) to prevent fetal movement and furosemide (3 mg/kg estimated fetal weight intravenously) to maximize venous capacitance immediately before the transfusion. Our goal was a final fetal hematocrit between 45% and 50%. Initially, hydropic fetuses were treated no differently than nonhydropic fetuses. Hydrops was defined as frank anasarca-edema and fluid accumulation in at least two cavities. The transfused blood was negative for cytomegalovirus, hepatitis B, and human immunodeficiency virus; compatible with mother and fetus; buffy coat poor; washed in saline solution to minimize the risk of viral contamination; irradiated to eliminate the risk of graft-versus-host disease; and resuspended in saline solution. Transfusions were initially repeated at 2- to 3week intervals when the hematocrit was anticipated to be between 25% and 30%, assuming a 120-day adult red blood cell lifespan. Frequent transfusions minimized both the duration of fetal anemia and the stimulus for fetal erythropoiesis.

The volume of blood infused was individualized for each fetus. We retrospectively tried several different formulas to estimate the volume necessary but found none were accurate enough. We therefore estimated the required volume on the basis of past experience, infused half the estimated requirement, and then measured the increase in the fetal hematocrit. By knowing the amount by which the hematocrit had increased after the first aliquot of blood, the total volume necessary to reach the desired final hematocrit could be easily calculated. In general, aliquots of 20 ml were used in fetuses <22 weeks, 30 ml for fetuses <26 weeks, 40 ml for fetuses <30 weeks, and 50 ml for fetuses <35 weeks. A transfusion consisted of two aliquots.

Laboratory tests monitored during transfusion included a complete blood cell count (Technicon, Tarrytown, N.Y.) and a Kleihauer-Betke stain to determine the percent circulatory fetal red blood cells. These were performed in the central hospital laboratory. Umbilical venous pH, Pco₂, and Po₂ were measured in heparinized plasma (Instrumentation Laboratory, Lexington, Mass.).

Data were maintained prospectively in a computerized data base. The results are presented as the mean ± SD unless stated. Analyses included paired and unpaired t tests, cross tabulation, and stepwise multiple regression. A $p \le 0.05$ was considered to indicate a significant difference.

Results

Forty-eight fetuses underwent 142 simple intrauterine intravenous transfusions for the treatment of fetal hemolytic anemia. The overall survival rate was 96%. Thirty-seven fetuses (77%) received two or more transfusions (maximum, seven).

The mean gestational age at the first transfusion was 28 ± 5 weeks (range, 18 to 36 weeks). The mean hematocrit before the first transfusion was 20% ± 8%

(range, 4% to 30%). In subsequent transfusions, the mean hematocrit before the procedure was $27\% \pm 6\%$. The mean volume of packed red blood cells administered was 81 ± 38 ml (maximum, 200 ml). The mean hematocrit at the end of the transfusion was $47\% \pm 5\%$. No transfusion attempt was unsuccessful.

Thirteen fetuses (27%) were hydropic when therapy was initiated. Although the hydrops resolved before delivery in all cases, the time required for complete resolution varied from 48 hours to 6 weeks. There were no deaths among nonhydropic fetuses, and the survival rate for hydropic fetuses was 85% (11/13). After the transfusion of 10 fetuses, the hematocrit was again measured 24 to 48 hours later. In all instances, this second hematocrit was within 3% of the final hematocrit obtained on completion of the transfusion.

Our practice was strongly affected by our early experiences. The first fetus transfused was delivered electively at 35 weeks, I week after its first transfusion. This neonate experienced severe hyperbilirubinemia in spite of multiple, double-volume exchange transfusions and is deaf. This episode strengthened our resolve to achieve a term delivery whenever possible. There were no other elective, preterm deliveries of treated fetuses.

Two hydropic fetuses died shortly after the first transfusion. Both of these cases led to a change in management. The first was an acidotic (umbilical venous pH = 7.31 before transfusion, 2 SDs from the norm = 7.39), hydropic 22-week fetus treated early in our experience (transfusion No. 26). The hematocrit was increased from 5% to 45%. One hour after the technically uneventful transfusion, a bradycardia developed and the fetus died shortly thereafter. There was no evidence of umbilical cord trauma at delivery 24 hours later. We assumed this death was secondary to volume overload, although we had not monitored intravascular pressure. We therefore added umbilical venous pressure measurement to our protocol for all subsequently treated fetuses.

We have recently come to believe that transfusionrelated acidemia also may be a factor in the death of the hydropic fetus. This impression originated with our second loss but was supported by the first. Although the umbilical venous pressure of this acidotic (umbilical venous pH = 7.34) 25-week fetus remained stable during the transfusion and the heart rate was normal, the acidemia became profound (umbilical venous pH = 7.26). A bradycardia developed 10 minutes after the transfusion was completed and did not recover; the fetus died. In light of preexisting symptomatic maternal respiratory compromise (chronic lung disease) and the moribund state of the fetus, an emergency cesarean section was not performed. There was no evidence of umbilical cord trauma at delivery 48 hours later.

We then modified our protocol for the first trans-

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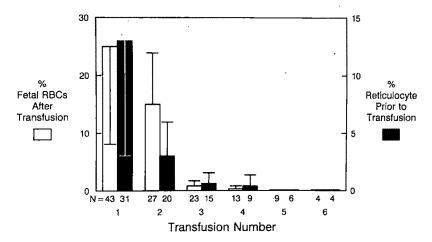


Fig. 1. Effect of intravascular transfusion on fetal reticulocyte count (percent before transfusion) and percent circulating fetal red blood cells (RBCs) (after transfusion) as estimated with Kleihauer-Betke stain.

fusion of hydropic fetuses to minimize the risks of volume overload and acidemia. The anemia is corrected in two steps 24 to 48 hours apart. The hematocrit is increased to approximately 25% (if the umbilical venous pressure is acceptable) during the first step and to 45% to 50% during the second step. Should the umbilical venous pH decline below 7.30, we infuse 4.2% sodium bicarbonate in 1 ml aliquots and recheck the pH 5 minutes later. The acidemia in three hydropic fetuses with a decline in umbilical venous pH <7.30 during transfusion has since been successfully treated with this protocol.

There were two nonlethal other complications directly related to the transfusion. In one woman whose fetus was seen initially with hydrops at 18 weeks, Staphylococcus epidermidis amnionitis developed several days after a transfusion performed at 25 weeks. She was delivered after intractable preterm labor. One woman was delivered at 35 weeks' gestation on an emergency basis because of a prolonged fetal bradycardia after inadvertent puncture of the umbilical artery before the transfusion was initiated. Puncture of the umbilical artery is a recognized risk for fetal bradycardia.4 These children are alive and well (18 and 43 months of age, respectively, at submission of manuscript). The overall incidence of bradycardia was 8%. Except for the one episode leading to cesarean delivery and the two described episodes associated with fetal death, each resolved within 10 minutes. All neonates who experienced a bradycardia and survived are said to be developing normally by either the pediatrician or a parent.

There were five obstetric complications not temporally related to transfusion. Three women experienced preterm premature rupture of membranes followed by preterm labor 1 and 3 weeks after the last of several transfusions. Two growth-retarded fetuses were deliv-

ered because of abnormal results of heart rate testing 2 to 3 weeks after the last transfuson. These five deliveries each occurred between 32 and 36 weeks and were associated with an uneventful neonatal course.

Simple intravascular transfusion was well tolerated by the fetus. Because the infused blood was acidotic (pH, 6.9 to 7.0), umbilical venous pH declined (7.40 \pm 0.02 to 7.35 \pm 0.03, p < 0.0001) during the transfusion. Severe fetal acidosis necessitating bicarbonate was seen only in fetuses with a hematocrit \leq 15%. Transfusion improved fetal oxygen delivery by increasing hematocrit; the umbilical venous PO₂ remained stable (39 \pm 7 to 40 \pm 6 mm Hg, p = 0.16).

In spite of the fairly large intravascular volume load, the umbilical venous pressure (corrected for amniotic fluid pressure) of nonhydropic fetuses rose only 5 ± 4 mm Hg (p < 0.0001) over baseline (range, 0 to 13 mm Hg). It remained within the normal range (<10 mm Hg) during 69% of procedures. Four of the five hydropic fetuses in whom umbilical venous pressure was measured had an elevated pressure before the first transfusion (range, 11 to 15 mm Hg). In three, the umbilical venous pressure rose abruptly (10 to 12 mm Hg) in spite of limiting the volume transfused to an amount adequate to raise the hematocrit to between 25% and 30%. When the umbilical venous pressure was measured again in these three fetuses just before the second transfusion 24 to 48 hours after the first, it had declined to a mean of 6 mm Hg (p = 0.07) and remained in the normal range throughout the second transfusion. In the remaining two hydropic fetuses studied, the umbilical venous pressure declined into the normal range during the course of the first transfusion. This sustained decline was not observed in any nonhydropic fetus.

The fetal reticulocyte count dropped after the ane-

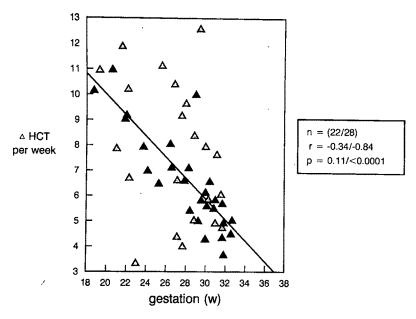


Fig. 2A. Effect of gestational age on decline in hematocrit after transfusion. *Open triangles*, Decline in hematocrit between first and second transfusions; *closed triangles*, decline for all subsequent transfusions. Regression line is based on all subsequent transfusions. *HCT*, Hematocrit.

mia had been corrected by transfusion. Within 3 weeks of the second transfusion, the mean reticulocyte count was <1% (Fig. 1). In addition, the percentage of circulating fetal red blood cells by the conclusion of the third transfusion was <1% (Fig. 1).

To determine with greater precision how often transfusions should be repeated, we calculated the rate the fetal hematocrit declined between transfusions performed January 1985 to October 1989 (32 fetuses, 91 transfusions) by subtracting the pretransfusion hematocrit from the final hematocrit of the previous transfusion and dividing the result by the intervening weeks. There was an inverse relationship between the rate the hematocrit declined and the gestational age at which the transfusion was performed (Fig. 2A). The scatter was quite wide for the decline in hematocrit per week between first and second transfusions (r = -0.34, p = 0.11). However, the correlation between hematocrit decline and gestational age for all subsequent procedures was very good (r = -0.84; p < 0.0001; standard error of the estimate 1.082). Fetal blood contributed minimally to the hematocrit after the second transfusion; therefore a differing rate of hemolysis for different populations of red blood cells cannot account for the close relationship between hematocrit decline and gestational age.

The relationship between gestational age and the rate of hematocrit decline was confirmed prospectively in the next 13 transfusions performed between November 1989 through mid-January 1990. The rate of decline in hematocrit between procedures closely approxi-

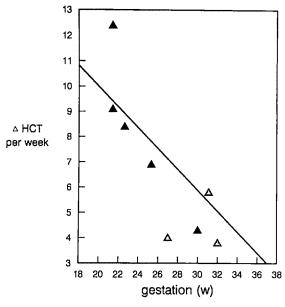


Fig. 2B. Prospective confirmation of relationship shown in Fig. 2A with new data. *Open triangles,* Decline in hematocrit between first and second transfusions; *closed triangles,* decline for all subsequent transfusions. Regression line is from Fig. 2A. *HCT,* Hematocrit.

mated the regression line obtained for the first data set (Fig. 2B). The gestational age influence on the rate of hematocrit decline after the second transfusion required a 2- to 3-week interval between transfusions at 20 weeks' gestation while allowing a 4- to 5-week interval after 32 weeks' gestation (assuming similar final hematocrits).

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Table I. Neonatal outcomes of fetuses treated with intravascular transfusion (N = 46)

	No.	%
Survival	46	96
Hydrops	13	85
Nonhydropic	35	100
Term delivery	32	74
Exchange transfusion		
0	35	76
1	8	17
>1	3	7
Simple transfusion		
Ô	33	72
1	7	15
>1	6	13

Table II. Neonatal clinical features of fetuses treated with intravascular transfusion

	Mean	SD
Gestation (wk)	36.7	2
Hematocrit at delivery (%)	33	8
Reticulocyte count (%)	2	3
Kleihauer-Betke (fetal red blood cells in umbilical cord smear)	10	17
Duration of phototherapy (hrs)	95	78
Intravascular transfusion	165*	101
>1 Intravascular transfusion	76	54
Double-volume exchange transfusion		
1 Intravascular transfusion	57.1†	
>1 Intravascular transfusion	20.7	
Hospital days (term delivery)		
1 Intravascular transfusion	8.6‡	6
>1 Intravascular transfusion	4.8	2

^{*}p < 0.0003.

Eight-two percent (36/44) of surviving neonates without heart rate abnormalities associated with growth retardation were delivered at term (Table I). Eleven of the 46 survivors (includes nonelective preterm delivery) (24%) underwent one or more double-volume exchange transfusions. Eight of the 11 required only one exchange. Two of the eight were performed at outside institutions per routine rather than as treatment for hyperbilirubinemia. Simple transfusion for treatment of anemia was performed after discharge in 13 neonates (28%). Each was associated with a delayed return of reticulocytes to the peripheral circulation rather than early neonatal hemolysis.

Neonates transfused more than once antenatally (and therefore delivered at least 9 weeks after transfusion therapy had begun) had a less complicated postnatal course than infants transfused only once (Table II). While not all data were available, they did receive less phototherapy and had a near-significant decrease in the likelihood of receiving a double-volume ex-

change transfusion. When delivered at term, neonates antenatally transfused more than once spent less time in the hospital. The mean peak total bilirubin was lower in term neonates whose reticulocyte count at birth was $\leq 1\%$ (10.8 \pm 6 mg/dl, n=8 vs 15.8 \pm 6 mg/dl, n=10; p<0.05). There was a trend toward reduced phototherapy when the amount of circulating fetal red blood cells was $\leq 5\%$ (61 \pm 59 hours, n=13 vs 133 \pm 151 hours, n=5; p=0.08).

As the number of patients with successful pregnancies grew over the 6-year period, we noticed a change in both patients' and referring physicians' attitudes toward subsequent pregnancies. Over the last 2 years, three women have chosen to undergo another pregnancy. In two of the three, the first treated fetus had presented with hydrops.

Comment

Our experience demonstrates that simple intravascular transfusion for the treatment of fetal hemolytic anemia can lead to an excellent perinatal outcome. It is safe in experienced hands and allows a term delivery in the absence of other obstetric indications.

Fetal anemia is the direct cause of many of the sequelae of hemolytic disease. The data suggest that the maintenance of the fetal hematocrit at near-normal levels throughout gestation accounted for the overall good neonatal outcome. The selection of a hematocrit that was actually <2.5th percentile for gestation as the threshold to initiate transfusion therapy was initially a pragmatic decision. Although we were reticent to begin therapy knowing that the fetus could tolerate a lower hematocrit well, we were unsure how rapidly a mild anemia might worsen. In hindsight, our selection appears to have been reasonable because several fetuses evaluated1 would have been transfused unnecessarily if the 2.5th percentile had been selected for the threshold, and hydrops developed in several high-risk fetuses with normal hematocrits over a 2-week interval.

The perinatal survival rate of 96% is similar to that in a slightly smaller series reported by Harman et al.5 (44 fetuses). However, this group performed their transfusions at closer intervals and delivered at earlier gestational ages. Although it was not our purpose to compare intravascular transfusion with intraperitoneal transfusion for the treatment of fetal hemolytic anemia, Harman et al. did. This is one of the few centers that has a large published experience with both intraabdominal and intravascular transfusion. They found an improved perinatal outcome with intravascular transfusion compared with intraperitoneal transfusion. Although another group reported a similar survival rate with a combination of amniotic fluid studies, intraperitoneal transfusion, and preterm delivery,6 this report seems an exception.5, 7-12 In addition, some fetuses

 $[\]dagger p = 0.06.$

p = 0.01.

transfused solely on the basis of amniotic fluid studies may not in fact need transfusion and thus may bias survival statistics in a favorable way. In a recent series of untreated isoimmunized pregnancies whose changes in optical density at 450 nm measurements were in zone III, Frigoletto et al. 13 noted that four of 11 fetuses (37%) had a hematocrit ≥30% at delivery and thus would not have qualified for transfusion by our criteria. Our experience14 with the relationship of the change in optical density at 450 nm to hematocrit is similar to that of Frigoletto et al.

In our study fetuses with hydrops had elevated umbilical venous pressures. We initially assumed this reflected obstructed venous return. However, the umbilical venous pressure characteristically fell into the normal range within 48 hours of the first transfusion. This argues against hepatomegaly and venous obstruction as a sole explanation for the increase. Rather it suggests that the increased umbilical venous pressure in hydrops is associated with myocardial dysfunction, perhaps as the result of tissue hypoxia associated with a critically low oxygen-carrying capacity. Transfusion would improve cardiac function by eliminating hypoxemia.

The only two losses were in severely hydropic fetuses. Each had a hematocrit <10% and a preexisting metabolic acidosis. In spite of the critical fetal condition, we do not feel these losses were inevitable. Each led to a modification in the transfusion protocol. The role of volume overload in the first loss is unclear and while fetal exchange transfusion would minimize the risk of volume overload it would not prevent the acidemia and would add greatly to the procedure time.

On the basis of the decline in fetal erythrocytes and reticulocytes, it would appear that fetal erythropoiesis can be suppressed within 6 weeks of initiating transfusion therapy. Alternatively, rapid hemolysis of erythroid progenitor cells on or before release from the marrow could account for this observation. However, the finding of erythroid hypoplasia in marrow aspirates of treated neonates argues against the latter possibility as a sole explanation.15 Further, we observed that neonatal hyperbilirubinemia was directly related to the reticulocyte count at birth and was more severe when only one antenatal transfusion had been performed. Although further study is needed, the 24% incidence of neonatal exchange transfusion in our series is less than that of neonates managed by either intraperitoneal transfusion or preterm delivery or both. 15, 16

Cordocentesis and intravascular transfusion are deceptively simple. In practice, they require an experienced team and a laboratory capable of performing a variety of tests on a small volume. Our series was drawn from a delivery base approaching 60,000 per year. It is not feasible from the standpoint of personnel and expense for every hospital to offer a similar service.

These are facilities that must be regionalized to maximize both safety and efficacy. The prior recommendation⁷ that a center perform ≥12 intraperitoneal transfusions per year or refer the patient to an experienced center is applicable to intravascular methodology.

In conclusion, treatment of fetal anemia by simple intrauterine intravascular transfusion is associated with a high survival rate, permits term delivery, and would appear to reduce immediate neonatal morbidity.

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Lack of an association between late fetal death and antiphospholipid antibody measurements in the second trimester

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To examine the relationship between elevated antiphospholipid antibody levels in the maternal circulation and late fetal death, we carried out a case-control study in which levels of anticardiolipin and antiphosphatidylserine antibodies were measured in banked second-trimester sera from 309 pregnancies ending in fetal death and from 618 viable control pregnancies. The sera were obtained from a population base of approximately 22,000 pregnancies enrolled for maternal α -fetoprotein screening between 15 and 20 weeks' gestation. The anticardiolipin immunoglobulin G level was markedly elevated (15.6 SD) in one serum sample associated with a fetal death. Otherwise, the anticardiolipin and antiphosphatidylserine measurements were similar in the two populations. Several other factors known to be associated with fetal death were also examined, and these all demonstrated the expected relationship. Antiphospholipid antibody measurements obtained at 15 weeks' gestation or later in the general pregnancy population are not helpful in identifying pregnancies at risk for fetal death. (AM J OBSTET GYNECOL 1991;165:1308-12.)

Key words: Antiphospholipid antibodies, second trimester, late fetal death, general pregnancy population

Elevated levels of antiphospholipid antibodies in blood (most prominently anticardiolipin and antiphosphatidylserine) are closely associated with lupus anticoagulant activity.1 The resultant hypercoagulable state can be assessed by measuring the partial thromboplastin time, which is lengthened as a result of the antiphospholipid antibody binding to negatively charged phospholipid molecules that participate in the prothrombin complex responsible for converting prothrombin to thrombin.24 Lupus anticoagulant activity was originally identified in patients with systemic lupus erythematosus, but now it is known to occur with other immunologic disorders and also as an isolated finding. In obstetrics, identifying lupus anticoagulant activity has become recognized as an important component in evaluating recurrent pregnancy loss1,4; it is estimated that 94% of pregnancies where the lupus anticoagulant is present will be nonviable, if untreated.⁵ In addition, two studies have reported elevated levels of anticardiolipin antibody in 13% and 40%, respectively, of patients being studied for unexplained recurrent pregnancy losses.6,7

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Reprint requests: James E. Haddow, MD, Foundation for Blood Research, P.O. Box 190, Scarborough, ME 04070-1090. 6/1/30701 To date, only one published study has examined lupus anticoagulant and antiphospholipid antibody in a general obstetric population.⁸ In that study, two of 737 consecutively enrolled patients (0.27%) were found to have evidence of the lupus anticoagulant (both also had elevated anticardiolipin levels), and both pregnancies ended in spontaneous abortions. Elevated anticardiolipin levels without lupus anticoagulant activity were identified in an additional 14 pregnancies, and these were associated with a high rate of fetal deaths, preterm deliveries, and intrauterine growth retardation

This study used a case-control design to examine further the relation between antiphospholipid antibody and fetal death in a general pregnancy population, measuring both anticardiolipin and antiphosphatidylserine antibodies in banked sera.

Patients and methods

Patients. Sera from all pregnant women in Maine enrolling for routine maternal serum α -fetoprotein screening between August 1983 and November 1986 were coded and stored at -20° C. Subsequently, outcomes were obtained for 97% of these pregnancies. From this bank, 309 sera were identified from pregnancies ending in a nonviable outcome (cases), and each of these was matched for gestational age with sera from two viable pregnancies (controls) obtained within 2 months of the corresponding case serum sample. Although we are not aware of published data that relate

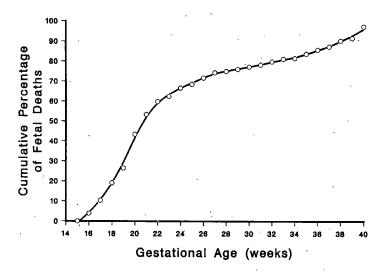


Fig. 1. Cumulative plot indicating time when fetal death was first identified in 309 cases.

to stability of antiphospholipid antibody in sera stored at -20° C, our own experience indicates that antiphospholipid antibody levels in serum samples with positive results remain stable for at least 6 years at that temperature. Fig. 1 is a cumulative plot of fetal deaths according to gestational age when first identified; two thirds of the deaths occurred by the twenty-fourth gestational week. All sera were obtained between 15 and 20 weeks' gestation, with a mean gestational age of 17.3 weeks. The samples were coded to protect confidentiality, blinded, organized into units of 33 case sera and 66 control sera, and submitted for analysis of antiphospholipid antibodies. Each unit of sera was thawed immediately before testing.

Antiphospholipid antibody enzyme-linked immunosorbent assay. Ninety-six-well microtiter plates (Immunolon I, Dynatech Laboratories, Alexandria, Va.) were prepared by diluting cardiolipin or phosphatidylserine (Sigma Chemical Co., St.Louis) in absolute ethanol to a final concentration of 45 µg/ml and adding 30 µl to each well. The solvent was allowed to evaporate overnight at room temperature in the dark. A total of 200 µl of assay buffer (10% fetal bovine sera in phosphate-buffered saline solution) was then added to each well; the plates were incubated at 25° C for 1 hour and then washed three times in phosphate-buffered saline solution with 1- to 2-minute incubations between washes. A total of 100 µl of a 1:100 dilution of each serum in assay buffer was added in duplicate, along with the calibrators and controls, and incubated for 1 hour at 25° C. The plates were then washed three times (as above), and 100 µl affinity-purified, Fc-specific, peroxidase-conjugated goat antihuman immunoglobulin G (IgG) (1:30,000 in assay buffer) or 100 µl affinitypurified, Fc5u-specific, peroxidase-conjugated goat antihuman immunoglobulin (IgM) (1:30,000 in assay buffer) (Jackson Immunoresearch, West Grove, Pa.)

was added and incubated at 25° C for 1 hour. The plates were then washed four times (as described), and color was developed by adding 100 µl tetramethylbenzidine and incubating for 4 minutes. Color development was stopped with the addition of 50 µl 0.06N hydrofluoric acid. The optical density was read at 650 nm on a Microplate Autoreader (BioTek, Winooski, Vt.). Spectrophotometric readings were adjusted by subtracting the readings observed on nonspecific binding plates (plates that did not receive phospholipid although they were processed in an identical fashion). The IgG assays were calibrated in antibody units with secondary reference standards prepared by serial dilution in assay buffer of a positive control containing 205 IgG phospholipid units (GPL).9 The IgM assays were calibrated similarly with a positive control containing 160 IgM phospholipid units (MPL).9

Data analysis. The population mean and standard deviation for each analyte was calculated after a log₁₀ transformation of antibody units. All individual results were then expressed in standard deviations above or below the mean. The 99th percentile of normal for each analyte was established by limiting the analysis to the 97% of control pregnancies with gestational age at delivery ≥37 weeks and a birth weight ≥2500 gm.

The Student t test for continuous and χ^2 test for discrete variables were the tests of significance for comparisons.

Results

Table I lists several comparative characteristics for the matched live births (controls) and fetal deaths (cases). Women in the two groups were of comparable age, and their serum samples were obtained at similar gestational ages. Years of education completed were lower among cases, and a greater proportion smoked

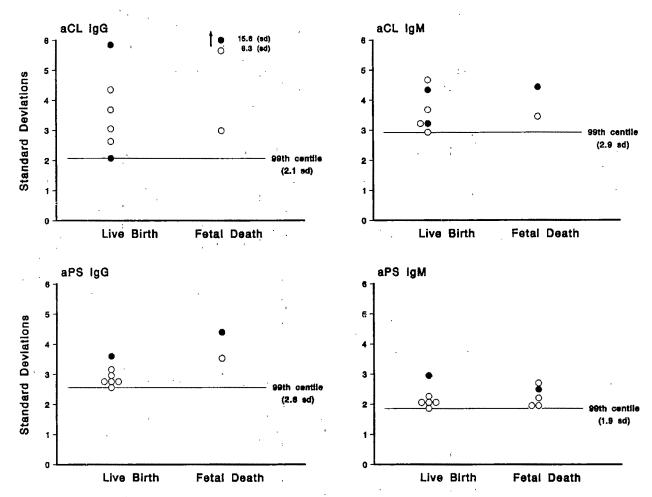


Fig. 2. Antiphospholipid antibody levels above 99th percentile of normal for 618 live births and 309 fetal deaths. Each circle represents serum antibody measurement from one individual, expressed as standard deviations above average of normal population. *Open circle*, Given individual's serum sample contains only one elevated antiphospholipid antibody concentration; *closed circle*, two antiphospholipid antibody concentrations are >99th percentile. Percents of antiphospholipid antibody values > 99th percentile in controls and cases for each antiphospholipid antibody type are as follows: anticardiolipin (*aCL*) IgG, 1% and 1%; anticardiolipin IgM, 1% and 0.6%; antiphosphatidylserine (*aPS*) IgG, 1% and 0.6%; antiphosphatidylserine IgM, 1% and 1.6%.

cigarettes, had suffered a previous spontaneous abortion, or experienced vaginal bleeding with the present pregnancy. Maternal serum α -fetoprotein values were also more often elevated in the cases than in the controls. All of these factors are widely recognized as being associated with fetal death.

Fig. 2 compares measurements of each of the four types of antiphospholipid antibody in the groups having live births or fetal deaths. For all four antiphospholipid antibody types, the proportions of measurements >99th percentile are similar in controls and cases, with only one outlying anticardiolipin IgG value (15.6 SD) associated with a fetal death.

Table II lists odds ratios for fetal death for each of the four antiphospholipid antibody types and compares them with four other factors recorded in the study population known to be associated with added risk for fetal death. An odds ratio >1.0 indicates that the risk for fetal death is higher when any given risk factor is present. Although the odds ratios for the four antiphospholipid antibody types vary from 0.57 to 1.67, none is significantly different from 1.0, as indicated by the 95% confidence intervals and p values. The limits specified for each of the four confidence intervals designate the range in which the true odds ratio lies. Therefore the upper limits of the confidence intervals indicate that it is unlikely that the true odds ratio is any higher than between 2.98 and 6.23, depending on the antiphospholipid antibody type measured. The odds ratios and confidence intervals are calculated with a 99th percentile cut-off, so that data from our study can be compared with those of an earlier published study. By contrast, the other four factors demonstrate significantly increased odds ratios consistent with the ex-

Table I. Selected characteristics of women whose pregnancies ended in fetal death (cases) or in live birth (controls)

Characteristic	Controls	Cases	p Value
No. of pregnancies	618	. 309	-
Age (yr)	$25.4 \pm 5.1*$	$25.8 \pm 5.5*$	0.4
Education (yr)	$13.1 \pm 2.1*$	$12.7 \pm 2.0*$	0.02
Gestational age at enrollment (wk)	$17.3 \pm 1.3*$	$17.3 \pm 1.3*$	0.9
Smokes cigarettes (%)	25.8	31.7	0.07
Previous spontaneous abortion (%)	27.1	36.3	0.007
Gravida ≥2 (%)	57.7	64.6	0.06
Vaginal bleeding, current pregnancy (%)	10.4	21.6	< 0.001
Maternal serum α-fetoprotein ≥2.0 multi- ples of median (%)	1.3	21	< 0.001

^{*}Mean ± SD.

Table II. Odds ratio for fetal death associated with elevated antiphospholipid antibody measurments and selected other risk factors

. Risk factor	Odds ratio	95% Confidence interval	p Value
Anticardiolipin IgG >99th percentile	1.00	0.20-4.50	0.73
Anticardiolipin IgM >99th percentile	0.80	0.15-4.63	0.90
Antiphosphatidylserine IgG >99th percentile	0.57	0.12-2.98	0.72
Antiphosphatidylserine IgM >99th percentile	1.67	0.44-6.23	0.60
Smoking	1.34	0.97-1.84	0.07
Previous spontaneous abortion	1.53	1.12-2.09	0.007
Vaginal bleeding	2.39	1.60-3.57	< 0.001
Maternal serum α-fetoprotein ≤2.0 multiples of median	28.2	10.7-80.8	< 0.001

pected association, with the exception of cigarette smoking, which falls just short of achieving statistical significance.

Comment

The only other available antiphospholipid antibody related information from the general obstetric population comes from a study at the Yale-New Haven Hospital obstetric clinic, reported by Lockwood et al.8 In that cohort study 737 sequential eligible patients were enrolled at an average of 16 weeks' gestation, and partial thromboplastin times and anticardiolipin IgG and IgM measurements were performed. Sixteen of the pregnancies were associated with anticardiolipin levels >99th percentile, and six of these (37%) ended in intrauterine deaths, as opposed to 40 (6%) out of the remaining 718 pregnancies. The Yale study's findings yielded an odds ratio of 6.0 for fetal death in the presence of an anticardiolipin measurement >99th percentile (95% confidence interval, 1.9 to 18.5) and therefore differ substantially from our data. A partial explanation for the different findings may result from a proportion of the samples from the Yale study having been obtained earlier in gestation, as indicated by three of the six fetal losses occurring before 13 weeks' gestation. The population served by the Yale-New Haven Clinic is at generally higher risk than the population in this study, as evidenced by the rates of low birth weight, cigarette smoking, and human immunodeficiency virus. Whether and how the generally high-risk characteristics of that population might relate to the higher rate of positive anticardiolipin results remain to be explained.

Fetal death after submission of a serum sample for maternal serum α-fetoprotein screening in our population occurs at a rate of 14 per 1000; thus the 309 samples associated with fetal death come from a population base of approximately 22,000 pregnancies. Only one of the 309 sera from pregnancies associated with fetal death was found to have an anticardiolipin IgG value sufficiently elevated to suggest the antiphospholipid antibody syndrome. It is likely, however, that evidence of this syndrome will be found more frequently early in pregnancy than indicated by this study, because most of the antiphospholipid antibody-associated fetal losses are likely to occur before the second trimester. This study demonstrates that, in the general pregnancy population, serum antiphospholipid antibody measurements (including anticardiolipin IgG and IgM and antiphosphatidylserine IgG and IgM) obtained during the second trimester are similar for pregnancies ending in subsequent fetal death or in live birth and thus appear not to be clinically useful.

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Severe ovarian hyperstimulation in a spontaneous singleton pregnancy

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Ovarian hyperstimulation syndrome in a spontaneous ovulatory cycle is exceedingly rare. We report a case of severe ovarian hyperstimulation associated with a spontaneous, otherwise normal singleton pregnancy. (Am J Obstet Gynecol 1991;165:1312-3.)

Key words: Ovarian hyperstimulation, CA 125, spontaneous ovulation

Ovarian hyperstimulation syndrome is a well-described clinical entity that usually represents the most serious complication associated with ovulation induction. Mild forms are infrequently associated with spontaneous ovulation and conception, primarily in the case of multiple gestations. A single case of severe ovarian hyperstimulation was reported in an anovulatory, non-pregnant patient who had trisomy 21 and was severely hypothyroid. We believe this to be the first report of a case of severe ovarian hyperstimulation associated with a spontaneously conceived singleton pregnancy in the absence of any medical problems and without evidence of trophoblastic disease.

Case report

A 16-year-old primigravid adolescent was seen with complaints of abdominal fullness, nausea, and shortness of breath of 2 days' duration. She described a 27-

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pound weight gain over the previous 2 weeks without any associated change in dietary habits. She had a history of regular, 28-day menses for the last 3 years, but she had had an 11-week period of amenorrhea. Vital signs demonstrated orthostatic hypotension. The result of physical examination was remarkable in that the abdomen was severely distended and diffusely tender but with normal bowel sounds and no signs of peritoneal irritation. She denied the recent use of medication and had no signs of hyperandrogenism.

Laboratory testing revealed an elevated β -subunit human chorionic gonadotropin level (279,000 mIU/ml; normal, <200,000), elevated progesterone level (214 ng/ml; normal, <200), hemoconcentration (hemoglobin, 17.2 gm/dl; normal, 11.2 to 16), and a slight hyponatremia (131 mEq/ml; normal, 132 to 140). Platelet and white blood cell counts were normal, as were blood urea nitrogen level, creatinine level, and coagulation profile. Ultrasonographic examination revealed a viable intrauterine pregnancy whose size was consistent with dates, a normal placenta, massive ascites, and bilaterally enlarged cystic ovaries measuring 12×16 cm on the right and 15×17 cm on the left. She was admitted with a diagnosis of ascites, bilateral ovarian cysts, and possible partial mole.

Additional testing was performed. These included a

normal result of chest x-ray examination, normal-appearing liver-spleen scan, normal results of thyroid and liver function tests, and normal results of Doppler flow studies of the inferior vena cava and portal venous systems. The CA 125 level (Centocor, Malvern, Pa.) was elevated at 1284 U/ml (normal, 0 to 35). A diagnostic paracentesis produced fluid that was compatible with an exudative process. Its cultures were negative, and no malignant cells were found. She was maintained at bed rest with fluid and sodium restrictions, yet she continued to complain of abdominal pain and difficulty breathing. She gained 7 pounds over the next 2 days. Because this was an undesired pregnancy and given the patient's symptoms and findings, a therapeutic abortion and paracentesis were performed.

The uterine contents demonstrated normal fetal tissues. A tissue cell culture from fetal parts revealed a normal female karyotype, 46,XX, in all 20 cells counted. Multiple sections of the placenta were microscopically reviewed and no hydropic changes or trophoblastic proliferation was noted.

Diagnosis was changed to severe ovarian hyperstimulation, and she was maintained solely on a regimen of enough intravenous fluids to maintain a urinary output. Diuresis began 10 days after the therapeutic abortion. Three weeks after operation she had lost 30 pounds, and a repeat ultrasonographic examination demonstrated resolution of ascites. The ovaries did not return to normal size until 3 weeks later. β-Subunit human chorionic gonadotropin and CA 125 levels both returned to normal nonpregnant values within this time period. Luteinizing hormone and follicle-stimulating hormone levels determined 6 months after operation were 12 and 8 U/L, respectively, and the menstrual cycle had resumed its regular monthly interval.

Comment

The complex clinical picture of severe ovarian hyperstimulation is an infrequent but closely watched for finding associated with ovulation induction and controlled ovarian hyperstimulation. Mild forms of ovarian hyperstimulation have been reported previously in spontaneous conceptions but never a case as severe as this. Likewise, this young woman had no signs or symptoms of chronic anovulation or hyperandrogenism, i.e., polycystic ovarian syndrome.

Partial moles are very difficult to diagnose. The finding of a normal karyotype from a fetal part does not rule out the possibility that triploidy may exist elsewhere in the placenta or fetus but does decrease this

CA 125 levels can be elevated in women in many processes that can irritate the peritoneum. These include endometriosis, infections, and certain epithelial (nonmucinous) tumors of the ovary. CA 125 is associated with coelomic epithelium and amnion during embryonic development, so mildly elevated CA 125 levels are an expected finding in normal and abnormal pregnancy. Elevated levels also have been described during ovulation induction and with ovarian hyperstimulation,2 but the levels reported here are much higher. Theoretically, pregnancy and severe ovarian hyperstimulation have combined to cause these increased levels of CA 125 noted in this case.

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Pseudoprognathism—An auxiliary ultrasonographic sign for transvaginal ultrasonographic diagnosis of cleft lip and palate in the early second trimester

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Cleft lip and palate have been diagnosed antenatally only in the late midtrimester or at later gestational ages, when it is too late for pregnancy termination. The diagnosis of fetal cleft lip was made in three low-risk nulligravid women at 14 to 16 weeks of gestation by systematic screening with a 6.5 MHz transvaginal transducer and was confirmed by postabortal examination. (AM J OBSTET GYNECOL 1991;165:1314-6.)

Key words: Cleft lip, cleft palate, pseudoprognathism, transvaginal ultrasonography, early pregnatal diagnosis

Three cases of cleft lip and palate were diagnosed at 14 to 16 weeks of gestation by a 6.5 MHz transvaginal transducer.

Case reports

Case 1. A 35-year-old, nulligravid woman was referred for routine ultrasonographic scanning at 14 weeks' gestation. Her medical history was unremarkable.

Ultrasonographic examination of the fetal face revealed a cleft lip to the left of the midline, clearly evident on tangential section of the upper lip (Fig. 1, A). A sagittal left paramedian section demonstrated protrusion of the mandible, pseudoprognathism (Fig. 1, B). No other fetal anomalies were detected.

After informed consent was given by the patient, the pregnancy was terminated at 18 weeks' gestation by intraamniotic instillation of 40 mg prostaglandin $F_{2\alpha}$. Examination of the abortus confirmed the cleft lip and palate (Fig. 1, C) and a single umbilical artery. No other malformations were detected.

Case 2. A 35-year-old, gravida 3, para 3 woman was referred for ultrasonographic screening at 14 weeks of her fourth gestation. Her history was remarkable for mental retardation of her older child, of unknown cause. No consanguinity, exposure to possible teratogens, or family history of congenital anomalies was recorded. Chorionic villus sampling, performed at 9 weeks of the current gestation because of maternal age,

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recorded a normal, 46,XY karyotype. On transvaginal ultrasonography, pseudoprognathism—protrusion of the mandible—was detected only on one sagittal, paramedian section of the fetal head, compatible with a cleft lip of 1 to 2 mm width (Fig. 2, B). Pregnancy termination was performed at 15 weeks 5 days, by intraamniotic instillation of prostaglandin $F_{2\alpha}$. The cleft lip was confirmed on postabortal examination of the fetus (Fig. 2, D) without any other malformations.

Case 3. A 29-year-old, gravida 2, para 2 woman was referred for routine ultrasonographic screening at 16 weeks' gestation. Her medical history was unremarkable. On transvaginal ultrasonography holoprosencephaly and cleft midline were detected. A cleft lip was clearly visualized on both longitudinal and tangential sections of the upper lip. A sagittal median transvaginal ultrasonography section of the fetal head revealed a pseudoprognathism compatible with the cleft midline lesion. After informed consent, termination of pregnancy was performed at 16 weeks, confirming the diagnosed fetal malformations.

Comment

In spite of decades of intensive research regarding one of the most common anomalies, cleft lip and palate, full knowledge of its cause and pathogenesis remains elusive. There seems to be both a genetic and an environmental component in the etiology of these disorders. The incidence varies between 1 per 1000 live birth in whites to 1.7% in Japan. In 75% to 80% of cases the cleft lip is unilateral, more on the left side, and in two thirds of cases it is associated with cleft palate. The anomaly is more prevalent in male fetuses, 2:1. The genetic etiology of cleft lip seems to be an autosomal recessive major gene with reduced penetration. By the fourteenth week of gestation, the facial contour of the developing fetus is virtually complete. Therefore detection of this anomaly should be at-

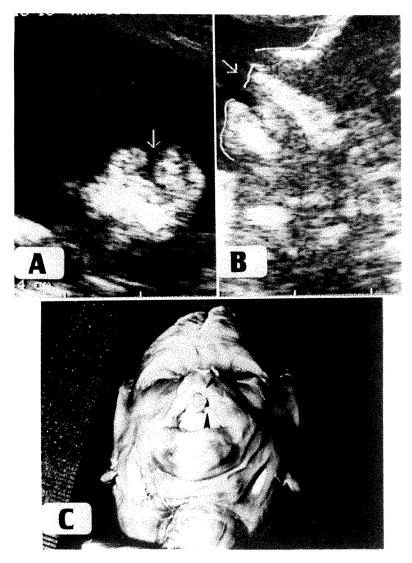


Fig. 1. A, Tangential transvaginal ultrasonographic section of upper lip demonstrating cleft lip (arrow) in case 1. B, Sagittal left paramedian section of same fetus demonstrating relative protrusion of mandible (pseudoprognathism) caused by cleft lip. C, Cleft lip (arrows) in aborted fetus (in case 1), confirming ultrasonographic diagnosis.

tempted as early as the fourteenth week, because cleft lip and palate may be associated with other fetal malformations in 7% to 13% of cases, some of them consisting of one of many syndromes.1

Prognathism, a relative protrusion of the mandible, is a rather common clinical occurrence. A total and an alveolar mandibular prognathism can be distinguished. In total mandibular prognathism the entire mental plate is obliquely inclined, but in alveolar prognathism only the alveolar process is inclined.

In contrast to the prognathism, which is usually caused by mandibular overgrowth, the pseudoprognathism that was detected by transvaginal ultrasonography in our three reported cases was seen in only one sagittal paramedian section (Figs. 1, B, and 2, B). The pseudoprognathism, or relative protrusion of the mandible as compared with the maxilla, could be detected only on the sagittal, paramedian section that passed through the cleft lip and palate. All the other sagittal sections of the same fetus did not show this protrusion because the maxilla and mandible were normally developed except in the area of the cleft lip and palate (Fig. 1, A). Therefore we suggest this ultrasonographic sign, clearly detected by us in the early second trimester in three cases of cleft lip, as an auxiliary ultrasonographic sign for the diagnosis of this fetal anomaly.

To the best of our knowledge, there was no description of fetal cleft lip or palate as early as 14 weeks' gestation.2 The early diagnosis allows for termination of pregnancy in cases where additional major malfor-

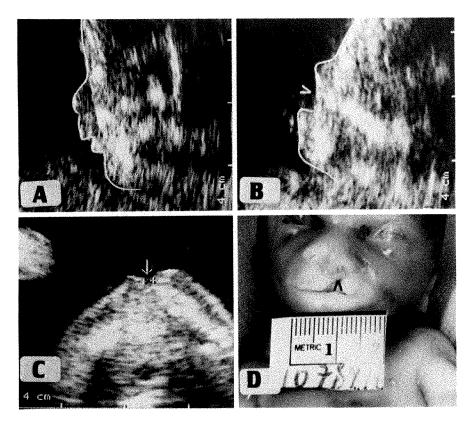


Fig. 2. A, Sagittal median transvaginal ultrasonographic section of fetus in case 2, compatible with normal facial profile (contour). **B,** Sagittal left paramedian section of same fetus, through cleft lip (arrow), showing relative protrusion of mandible (pseudoprognathism). **C,** Tangential section of upper lip, demonstrating cleft lip (arrow). **D,** Cleft lip (arrow) in aborted fetus.

mations are diagnosed, after informed consent of the parents. In cases where parental choice was to continue the pregnancy, early detection at 14 to 16 weeks may allow for karyotype assessment by amniocentesis at 16 weeks. In families with previously affected children (by cleft lip and palate), visualization of normal facial contour may be reassuring for the anxious parents. This is also pertinent for previous fetal exposure to possible teratogens such as hydantoin or high doses of glucocorticosteroids.¹

We conclude that early second-trimester detection of cleft lip by transvaginal ultrasonography enables informed parental choice of continuing or terminating the affected pregnancy.

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Extended longitudinal study of uterine activity among low-risk women

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Eighty-nine black women without major medical risks for preterm labor participated in this longitudinal, prospective study for the evaluation of usual prelabor uterine activity and for the assessment of how gestational age, time of day, maternal weight, age, and parity affected contraction frequency. Participants wore an ambulatory tocodynamometer for 72 consecutive hours at three points in gestation during the second and early third trimesters while engaged in usual home and work activity. Data obtained from the 81 women with uncomplicated term pregnancies demonstrated a significant increase in contraction frequency with advancing gestational age between 22 and 33 weeks. After 26 weeks, significantly more uterine activity occurred at night. Perhaps because of detection difficulties, contraction frequency was inversely related to maternal weight in women weighing >112% ideal weight. Maternal age and parity did not affect contraction frequency. Contraction characteristics of eight women experiencing preterm labor or medically indicated preterm delivery are described. (AM J OBSTET GYNECOL 1991;165:1317-22.)

Key words: Normal uterine contractility, uterine contractility in pregnancy, tocodynamometry, diurnal rhythms

The recent introduction of ambulatory uterine activity monitoring has refocused obstetric interest on the importance of uterine contractions that occur throughout the second and third trimesters of pregnancy ("prelabor" contractions).1 Limited information is available about the normal range of prelabor uterine contractions, especially those occurring outside a hospital setting in women without major medical risks for preterm labor.2,3 An understanding of these phenomena would provide useful information for surveillance of normal pregnancies and for comparison with high-risk patients. In addition, tocodynamometry data from lowrisk women in home and work settings can be used to evaluate the effects of selected maternal factors (such as age, parity, and weight) on uterine contraction characteristics. To date, most studies have been done in women considered to be at high risk for preterm labor and have been directed toward achieving an early diagnosis of preterm labor. This is the first study to serially monitor medically low-risk women over several 24-hour periods outside the hospital.

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Material and methods

Study population. In this prospective cohort study, 89 women were monitored for 72 hours at 4-week intervals at three points in gestation (weeks 22 to 25, 26 to 29, and 30 to 33). Participants were recruited by the study coordinator from the routine obstetric clinics of the hospital of the University of Pennsylvania between September 1986 and July 1987. All black women who presented for prenatal care before 24 weeks' gestation were approached and approximately one third agreed to participate in the study. The main reasons for refusal were concerns about equipment safety, inconvenience of study design, or lack of stable home environment. Women completing the study received \$10 per day monitored. Women with major medical risks for preterm labor (multiple gestation, history of preterm labor or delivery, and uterine malformation), were excluded, as were women with known substance abuse history, major psychiatric disease, and illiteracy. The study was approved by the Human Research Committee at the University of Pennsylvania.

The participants were all between the ages of 18 and 38 years (mean age, 24.1 ± 4.5 years) and 38% were nulliparous. These participant characteristics were similar to those of the overall clinic population.⁴ All participants explicitly denied cocaine or narcotic use, although urine and blood toxicology screens were not performed. (Substance abuse was not a widespread problem in our clinic population at the time.) For purposes of the study body weight was recorded as percentage of ideal weight for height and gestational age.⁵ Body weights varied widely: 16% were <90% of ideal weight, 32% were between 91% and 110%, 36% were

between 111% and 140%, and 16% weighed >140% of ideal weight. Eighty-one participants experienced uncomplicated term pregnancies, five had preterm labor, and three were delivered before 37 weeks' gestation because of medical complications (two with preterm premature rupture of the membranes, one with severe preeclampsia).

Determination of gestational age and preterm labor.

A clinically derived estimated delivery date was determined for each participant on the basis of last menstrual period and confirmed by first clinical estimate of size and first fetoscopic detection of heart tones. In addition, all patients had an ultrasonographic estimate of gestational age determined before 24 weeks of pregnancy. In 94% of cases, the ultrasonographic and clinical dates differed by <10 days, and in these cases the clinical dates were used. In five cases, when the dating disparity was greater, the ultrasonographic dates were used. To be diagnosed as having preterm labor, women needed to present with frequent uterine activity (minimum of four contractions in 20 minutes or eight contractions in 1 hour), as well as either cervical dilatation of ≥ 2 cm or effacement of $\geq 75\%$, or be observed to experience progressive cervical change. Patients not meeting these criteria did not receive tocolytics.

Uterine contraction monitoring. Participants were monitored during usual home and work activity. They were asked to wear a Termguard tocodynamometer for 72 hours, beginning on Monday evening of each study period. They were called by the study staff every 12 hours to transmit the uterine activity data by telephone. Monitoring was limited to weekdays to minimize variability that could be introduced by inclusion of different weekend activities. Compliance with the study was very good, with a mean of 61 hours of reliable contraction data per 72-hour study period (85%) obtained for interpretation and analysis.

The uterine monitoring strips were interpreted by one investigator, who was blinded to major study hypotheses, before the pregnancy outcome was known. Contractions were defined as smooth, symmetric deviations from baseline uterine tonus lasting at least 35 seconds and reaching a minimum of 2 mm in height. Intraobserver reliability was assessed by blinded rereading of a randomly selected 600 hours of uterine activity data. In 79% of the cases uterine contractions were identified identically, and in 95% of cases mean hourly contraction rates varied by one contraction per hour or less.

Data analysis. A descriptive analysis was undertaken to characterize uterine activity. Contraction frequency, intensity, and duration were recorded separately for each woman for each hour of each gestational age period monitored. Data for each individual were then combined and averaged to obtain mean hourly contraction rates and mean contraction intensity and duration for each of the three gestational age periods.

Values for all individuals with uncomplicated term gestations were combined to create summary estimates of contraction frequency, intensity, and duration for each gestational age period. Summary measures of contraction frequency, duration, and intensity were also assessed for specified time intervals within each gestational period, including each 24-hour period and two 12-hour periods (8 AM to 7:59 PM and 8 PM to 7:59 AM) for an averaged 24-hour day.

In the term, uncomplicated pregnancy group, a repeated-measures analysis of variance was used to examine whether contraction frequency (and likewise intensity and/or duration) significantly varied over the three gestational age periods, the 3 days within each study period, and the day versus night 12-hour intervals within each study period.6 The effects of three maternal variables (age, parity, and weight) on these contraction characteristics also were evaluated in the context of these models. Since the distribution of the contraction characteristics was skewed, natural logarithms were used to normalize the data. In addition, cosine analysis with a repeat period of 24 hours was applied to each individual's 72 hours of hourly contraction rates to determine if there were consistent diurnal rhythms.7

Multiple linear regression modeling also was used to assess the relationship between maternal weight and uterine contraction characteristics for each gestational age period. To determine whether there was a threshold to the apparent weight effect, piecewise regression analysis was performed to examine the relationship between weight and contraction frequency in different weight strata.8

Results

Uncomplicated pregnancies: Gestational age effects. In the group of 81 women with term, uncomplicated pregnancies, the average hourly contraction rate increased significantly with advancing gestational age (p = 0.0001 by repeated-measures analysis) (Table 1). When maternal weight, age, and parity were included in the model, the association between gestational age and contraction frequency remained significant (p = 0.01). Mean *intensity* of uterine contractions also increased significantly with increasing gestational age (p = 0.0001) (Table I). This effect remained significant even when maternal weight, parity, and age were included in the model (p = 0.03). In contrast, contraction duration did not change significantly with gestational age (Table I).

Uncomplicated pregnancies: Diurnal effects. On the basis of our review of the literature, we anticipated increased uterine contraction frequency in the evening and night hours as compared with the day hours. To test this hypothesis, we created two 12-hour data sets for each gestational age period, one consisting of all data recorded between 8 AM and 7:59 PM (day) and the

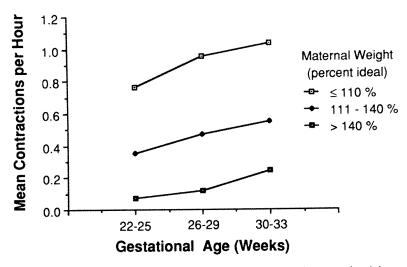


Fig. 1. Mean hourly contraction frequency by gestational age and maternal weight strata.

other contained all data recorded between 8 PM and 7:59 AM (evening and night). During the first gestational age period, the mean \pm SD hourly contraction rate for daytime hours was 0.39 ± 0.55 and the evening-night hourly rate was 0.57 ± 0.68 ; during the second period, 0.49 ± 0.64 and 0.76 ± 0.77 ; and during the third period, 0.62 ± 0.76 and 0.85 ± 0.84 , respectively. Repeated-measures analysis, controlling for maternal weight, was performed for each gestational age period. In each analysis a significant diurnal pattern was noted (p = 0.03 for the first, p = 0.001 for the second, and p = 0.02 for the third period). When maternal weight, age, parity, and sexual intercourse were included, the repeated-measures analysis remained significant only for the second (p = 0.002) and third (p = 0.003) periods.

To evaluate the regularity of the nocturnal increase in uterine contraction frequency, a cosine analysis was performed with the use of each participant's hourly contraction rate for each of the 72 hours monitored for each gestational age period studied. The purpose of this analysis was to determine if there was a 24-hour cyclicity to the individual uterine activity data. A correlation between the cosine curve and the individual's data points was required to reach a p value of <0.05 to be considered a significant fit. During the first gestational age period, 15.5% of the fits were significant. During the second gestational age period, 17.6% of the fits were significant. The percentage of significant fits was much higher in the third gestational age period at 39.7%.

Uncomplicated pregnancies: Effects of maternal weight, age, and parity. Maternal weight also had a clear relationship to uterine contraction frequency (p = 0.0001). As shown in Fig. 1, the mean hourly contraction rates for each of three maternal weight categories (≤110%, 111% to 140%, and >140% of ideal weight) demonstrate parallel increases with increasing

Table I. Contraction height and duration by gestational age period for term participants

	Gestational age				
	22-25 wk	26-29 wk	30-33 wk		
Contractions					
(No./hr)					
Mean ± SD	0.49 ± 0.57		0.73 ± 0.70		
Range	0.01 - 2.71	0.01 - 3.19	0.01 - 4.91		
Height (box					
height)					
Mean ± SD	0.71 ± 0.19	0.80 ± 0.24	0.88 ± 0.22		
Range	0.38 - 1.33	0.38 - 2.13	0.50 - 1.45		
Duration of					
contractions					
(sec)					
	61.6 ± 11.1	64.6 ± 8.3	66.7 ± 9.5		
	42.0 - 120.0	46.7 - 80.7	49.0 - 89.9		
Range	42.0 - 120.0	10.7 - 00.7	1010 0000		

gestational age. Multiple linear regression modeling revealed an inverse relationship between maternal weight and contraction frequency that was similar for all three gestational age periods evaluated (p = 0.0001 for each gestational age). Because uterine contractions increase with gestational age and there was a range of 4 weeks for each gestational age period, we evaluated whether the increased uterine activity observed in thin women could be due to their enrollment at later gestational ages. Therefore we evaluated the relationship between maternal weight and gestational age at entry in the study and found that gestational age at enrollment was completely independent of maternal weight.

In a study reported by Nageotte et al.,9 women who were delivered after 42 weeks' gestation had lower contraction rates at some given point in gestation than women who were delivered at term. We next looked at maternal weight with respect to gestational age at delivery and found no relationship. Finally, using multivariate regression analysis, we evaluated the relation-

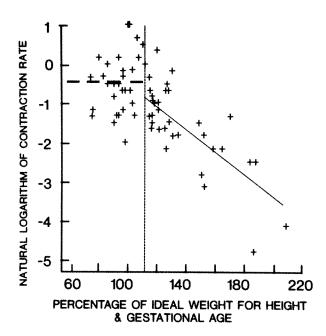


Fig. 2. Piecewise regression of contraction rate and percentage ideal maternal weight with threshold of 112% ideal weight. Regression line at <112% is nonsignificant (p=0.72) whereas that >112% is highly significant (p=0.0001). Data from second study period are presented.

ship of maternal weight on uterine contraction frequency, controlling for age and parity. The relationship between maternal weight and contraction rate remained highly significant for each gestational age period (p = 0.0001).

Because identification of a threshold above which uterine contraction rates are significantly related to maternal weight would be useful clinically, we evaluated our data in search of such a threshold. We used piecewise regression analysis, varying the weight cutoffs by percentage point between 110% and 125% of ideal maternal weight. We found that at <112% there was no significant relationship between maternal weight and contraction frequency in any of the three gestational age periods studied. When maternal weight was >112% ideal weight, there was a highly significant, inverse relationship between maternal weight and contraction frequency in all three gestational age periods studied (see Fig. 2). Each individual's percentage ideal body weight during the second study period was used throughout the analysis, although people varied minimally from one study period to the next with respect to this variable. All patients above the determined threshold remained so in all three study periods.

Wilcoxon-Mann-Whitney, Student *t*, and multivariate analyses were performed to determine whether maternal age or parity was related to contraction frequency, intensity, or duration. None of these maternal factors demonstrated any significant effects on uterine activity in our sample, nor did maternal weight have

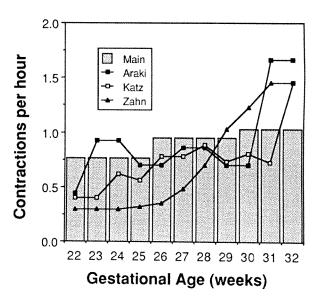


Fig. 3. Comparison of published data on contraction frequency by gestational age with current study results.

any independent relationship to contraction intensity or duration.

Complicated pregnancies. The primary purpose of this study is to provide descriptive data from low-risk women who were delivered at term. However, 8 of the 89 women in this study experienced either preterm labor (n = 5) or preterm birth without preterm labor (n = 3). Individual data for these women with complicated pregnancies including their percentages of ideal weight are shown in Table II. Although a number of results are above the mean for a given weight category and gestational age period, they do not appear to be out of the general range of values obtained from the 81 participants with uncomplicated pregnancies.

Comment

Contraction frequency. This study expands current knowledge about uterine contraction activity in the home and work setting among women with low-risk pregnancies. We have found only two other published studies dealing with uterine activity in ambulatory, lowrisk women.2.3 Zahn,2 using a telemetry device, monitored 26 nulliparous and 28 multiparous German women for 1 hour each day, between 25 weeks' gestation and delivery. Araki,3 using a portable device, provided cross-sectional data based on monitoring Japanese women at home for 30 minutes. The mean hourly contraction frequencies described by these investigators are quite similar to our more extensive data. In a study by Newman et al.10 of women at high risk for preterm labor, the uterine activity data for the 22 women with singleton pregnancies who did not have preterm labor are also similar to our data and those of Zahn and Araki (Fig. 3).

Table II. Contraction characteristics by gestational age period for women experiencing preterm labor or delivery

Case No.	Ideal weight	Mean frequency (No./hr)	Mean intensity (box height)	Mean duration (sec)
First gestatio	onal age period			
1	90	2.31	0.94	68.1
2	103	0.08	0.60	60.0
2 3	128			_
4	140	0.21	0.90	68.5
5	154	0.03	0.13	75.0
6	96	0.23	0.67	72.0
7	101	1.18	0.98	65.0
8	101	0.12	1.04	83.0
Second gesta	utional age period			
1		0.72	0.90	75.0
2		0.27	0.52	64.7
$\frac{2}{3}$		0.43	0.46	67.9
6		0.16	0.59	64.4
7		1.25	0.90	68.9
Third gestat	tional age period			
1	· .	0.39	0.90	82.7
2		0.65	0.61	61.0

Note: Cases 1 to 5 were complicated by preterm labor and 6 to 8 by preterm premature rupture of the membranes or preeclampsia with abruptio placentae.

Other investigators have demonstrated an increased frequency of uterine contractions in women with preterm labor. 1, 3, 9, 10 This increase is often apparent for several weeks but becomes more pronounced during the 3 days preceding labor. Our study was designed to evaluate contraction characteristics in low-risk women who were delivered at term and not to assess the relationship between uterine contraction frequency and preterm labor. Thus the lack of a statistically significant difference in contraction rates in our study is not unexpected given the very small number of women with preterm labor and the wide spacing of monitoring periods (every 4 weeks).

Diurnal patterns of contraction frequency. Pregnant rhesus monkeys display a consistent circadian oscillation in contraction frequency in the latter part of pregnancy. The time of onset of peak contraction activity is around 7 PM with the onset of darkness.11 The activity reaches a maximum at 10 to 11 PM. These changes in contraction frequency are seen only in pregnancies with a live fetus. Increased uterine contractions at night are frequently observed in pregnant women with preterm labor.12 Analyses of large series of births also demonstrate higher rates of onset of spontaneous labor during the evening and night hours.13.14

However, other data about diurnal variations in prelabor contractions in human pregnancies are relatively limited. Zahn² studied 57 women between 32 and 41 weeks' gestation, presumably for one 24-hour period, while they were hospitalized for unspecified reasons. He noted a diurnal pattern similar to that seen in the rhesus monkey with a peak contraction frequency increase of >50% over the mean between 10:30 PM and 2 AM. However, this report provides no information on the variability among patients or the effect of gestational age on this pattern. Clearly, uterine activity in hospitalized patients who are monitored in the supine position may not accurately reflect contraction frequency in normally active pregnant women. Arakis monitored 22 Japanese women at different points in pregnancy, during both day and night hours. He found a fluctuating pattern that had no consistent relationship to gestational age. Contraction frequency increased at night in 38% of these women and decreased or remained unchanged at night in the other 62%.

Our findings of increased uterine activity at night in the last two study periods are not inconsistent with the limited information provided above. Although more women than expected by chance (particularly in the third gestational age period) had a 24-hour cyclicity of contraction frequency by cosine analysis, the majority of women did not. This is not surprising when one considers the many variables in a participant's daily schedule, including coitus and physical and emotional state.

Obesity. Although not described in the literature, it will not surprise many obstetricians that fewer contractions were detected in obese women. First, it is often difficult to palpate contractions and to obtain adequate monitoring data by means of standard labor and delivery equipment in obese women. Second, extremes in maternal weight are thought to influence pregnancy duration. In particular, very malnourished women are at increased risk for preterm deliveries.15 Examining the other weight extreme, one recent case-control study found a 16.4% rate of pregnancies lasting >42 weeks' gestation in women who weighed >250 pounds as compared with a 4.6% rate in controls who weighed <200 pounds. However, most studies that have examined the relationship between maternal weight (controlled for maternal height) and duration of gestation across a wide range of maternal weights have found, at best, very weak associations. In our current, relatively small sample of women delivered at ≥37 weeks' gestation, no association was present between ideal maternal body weight and pregnancy duration.

Inasmuch as there were no maternal weight-related differences with respect to pregnancy duration in this study, it is likely that the inverse relationship between maternal weight and contraction frequency in the heavier women reflects technical difficulty in monitoring increasingly obese women. The Termguard I device emits a warning signal until the tocodynamometer is applied against the abdomen with a minimum of 8 mm Hg baseline pressure. Although it is possible to increase the minimum baseline pressure to 12 mm Hg, this was not done in the current study. Such an approach might have enhanced the detection of contractions in the more obese participants. Further work to confirm the appropriate maternal weight threshold and evaluate the effect of belt tightening for obese patients would be useful. It is reassuring that we found no relationship between maternal weight and contraction frequency for women weighing ≤112% ideal weight for height and gestational age because most pregnant women remain in this normal to slightly obese range.

Parity. Like Zahn, we found no effect of parity on the frequency of prelabor contractions. This is of interest because during active labor, parity appears to influence the frequency of contractions. ¹⁸ When uterine contractions during labor are measured by internal pressure catheters, the parous uterus expended significantly less energy to reach complete dilation as compared with the nulliparous uterus.

In conclusion, our study presents the first extensive longitudinal observation of uterine contractility in pregnant women monitored in their own environments. Prelabor contractions increase significantly with increasing gestational age but remain at levels well below those considered abnormal. More uterine contractions were noted during the evening and night hours than during the day hours. Obese patients weighing >112% ideal weight have a significant inverse relationship between maternal weight and detected uterine activity. Finally, maternal age and parity do not appear

to influence uterine contractility before the onset of labor.

We thank Richard Wentworth, PhD, Susan Jenkins, and Peter Nathanielsz, MD, PhD, for performing the cosinor analysis presented in this paper. We also acknowledge the close cooperation and technical support provided by the Bell of Pennsylvania Company and by Tokos Medical Corporation, which were critical to the success of this study.

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Videolaseroscopy for oophorectomy

Farr Nezhat, MD, Camran Nezhat, MD, and Sheryl L. Silfen, MD Atlanta, Georgia

Laparoscopic cophorectomy was performed on 94 ovaries in 76 patients. Indications included recurrent pain associated with endometriosis and adhesions in 17 patients (18 ovaries), ovarian endometriomas in 40 patients (40 ovaries), prophylactic cophorectomy (breast cancer) in one patient (2 ovaries), removal of the ovaries at the time of laparoscopic assisted vaginal hysterectomy in 15 patients (30 ovaries), and other indications in three patients (four ovaries). (AM J OBSTET GYNECOL 1991;165:1323-30.)

Key words: Operative laparoscopy, oophorectomy, videolaseroscopy, avoiding laparotomy, laparoscopy

Operative laparoscopy has been shown to be a safe, useful, and cost-effective alternative to laparotomy in cases of benign pelvic disease. 1-10 An increasingly wider variety of procedures are becoming possible, when performed by experienced operative laparoscopists.

Oophorectomy at laparoscopy with the use of endoloop suture has been previously reported.³ This report presents our experience with an alternative technique, videolaseroscopy (a combination of high-resolution video imaging and high-power carbon dioxide laser applied to operative laparoscopy) and electrocoagulation, to accomplish 94 consecutive oophorectomies.

Material and methods

All operations were performed by two surgeons (C.N. and F.N.), who have a combined experience of >5000 operative laparoscopies. All patients were seen in a private, largely referral clinical practice and operated on in an outpatient surgical suite of a large suburban hospital. From July 1987 to June 1990, laparoscopic oophorectomy, with videolaseroscopy techniques only, was performed on 94 ovaries in 76 patients ranging in age from 32 to 60. Indications were recurrent pain associated with endometriosis or pelvic adhesive disease, ovarian endometrioma (6 to 15 cm diameter), persistent ovarian cyst, prophylactic oophorectomy in breast cancer, and oophorectomy at the time of laparoscopically assisted vaginal hysterectomy; all patients undergoing laparoscopically assisted vaginal

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Table I. Indications for oophorectomy in 76 women

Indications	No. of patients	No. of ovaries	Age of patient (yr)
Endometrioma	40	40	32-43
Severe endometriosis and adhesions	17	18	36-49
Laparoscopically assisted vaginal hysterectomy	15 .	30	39-47
Ovarian cyst	3	4	32-52
Prophylactic oopho- rectomy	1	2	42
TOTAL	76	94	

hysterectomy had a history of endometriosis, adhesions, or leiomyomas, which precluded straightforward vaginal hysterectomy. Forty-eight patients had an intact uterus, whereas 28 had had hysterectomy. Tables I, II, and III summarize the clinical data regarding these 76 patients.

All patients with endometriosis or adhesions had previous medical or conservative surgical management that failed to relieve pain. The history was reviewed preoperatively, including previous operative reports, and pelvic examination and ultrasonographic examination were performed. Cases with unilocular ovarian cysts were initially managed by suppressive therapy with 50 µg estinyl estradiol-containing oral contraceptives or danazol. CA 125 levels were obtained for all patients beginning in 1988. Intraoperatively, the pelvis, upper abdomen, and diaphragmatic surfaces were inspected for signs of malignancy, and pelvic washings were obtained for cytologic testing. The cyst was then aspirated, and the fluid was examined grossly and sent for cytologic examination. The cyst wall was then opened and inspected for excrescences or irregular thickenings. Frozen-section histologic examination was done if, in

Table II. Oophorectomy in 48 women with intact uteri

	No. of patients	No. of ovaries
Laparoscopically assisted vaginal hysterectomy	15	30
Endometrioma	29	29
Breast cancer	. 1	2
Persistent functional cysts	I	2
Serous cystadenoma	1	1
Dermoid cyst	1	1
TOTAL	48	65

Table III. Oophorectomy in 28 women after hysterectomy

	No. of patients	No. of ovaries
Endometriosis and adhesions	17	18
Endometrioma	11	11
TOTAL	28	29

the judgment of the surgeon, the appearance was suspicious. All tissue obtained was submitted for permanent-section histologic examination.

All procedures were performed with the patient under general endotracheal anesthesia. Multiple abdominal punctures were used to introduce the laparoscope and suprapubic instruments. A miniature laparoscopemounted video camera with a high-resolution video monitor was used, allowing the operative field to be magnified and viewed by all operating room personnel and a more comfortable, upright position for the surgeon (Fig. 1). All procedures were video recorded to retain a permanent record on each patient. The carbon dioxide laser was introduced into the peritoneal cavity through the operating channel of the laparoscope with direct lens coupler and set at 30 to 80 W of power in superpulse mode for the purpose of cutting.5, 9-12 Bipolar forceps were introduced through a suprapubic portal and used for coagulation.

After the pelvis and abdomen were explored, oophorectomy was carried out in the following manner: Adhesions between the ovary and adjacent organs, pelvic walls, and broad ligament were lysed with a carbon dioxide laser. A Nezhat suction-irrigator probe (Cabot Medical, Langhorne, Pa.) and later a Nezhat-Dorsey probe (Karl Storz, Culver City, Calif.), introduced suprapubically, were used as a backstop and to provide constant suction, irrigation, and smoke evacuation. When the ovary was adherent to the lateral pelvic wall and in cases with previous hysterectomy, the technique of hydrodissection¹² was used to open the peritoneum, beginning at the pelvic brim, and to identify the course of the ureter and major blood vessels. With the same techniques, the descending colon and rectosigmoid colon were dissected from the site of the left infundibulopelvic ligament and ovary. Ovarian cysts were then aspirated, allowing easier handling of the deflated cyst and smaller ovary. Once the ovary was completely mobilized, it was held under tension with a grasping forceps, and the ovarian ligament was coagulated with bipolar cautery and transected with the laser at its junction to the uterus (Fig. 2). With 20 to 25 W, the cautery was applied briefly, desiccating and blanching but not overdesiccating the tissue, to reduce the blood flow to the pedicle to be transected. The mesovarium was then serially blanched, coagulated, and transected at 1 to 2 cm increments, working from medial to lateral, until the ovary was removed (Figs. 3 to 5). When the ipsilateral fallopian tube also was removed, the isthmic portion of the tube was severed and incised along with the ovarian ligament (Fig. 6); after identification of the ureter, the infundibulopelvic ligament was coagulated and transected at 1 to 2 cm increments, working from lateral to medial, until the adnexa were removed as described above (Figs. 7 and 8).

In most cases the ovary was removed from the peritoneal cavity through a 10 mm trocar sleeve placed in one of the suprapubic puncture sites. The ovary was grasped with a forceps, and then forceps and sleeve were removed together, delivering the ovarian tissue to the abdominal wall, where it was then grasped by a Kelly clamp and removed. In 25 patients (one with serous cystadenoma, nine with endometriomas associated with myomectomy, and 15 with laparoscopically assisted vaginal hysterectomy) the tissue was removed by posterior colpotomy.13 After removal of the ovary, thorough irrigation of the pelvic cavity was performed. The pedicles were then inspected under the water, both in the presence of a pneumoperitoneum and again after carbon dioxide evacuation, to ensure hemostasis after intraperitoneal pressure was reduced.

Results

Laparoscopic oophorectomy with the technique of videolaseroscopy was performed on 94 ovaries in 76 patients. The procedure was completed in each attempted case. Blood loss during oophorectomy was minimal. No major intraoperative or postoperative complications occurred, and no long-term complications have developed. No ovarian malignancies were encountered in this group of patients. Minor complications were limited to abdominal wall ecchymosis (two patients) and severe shoulder pain (nine patients).

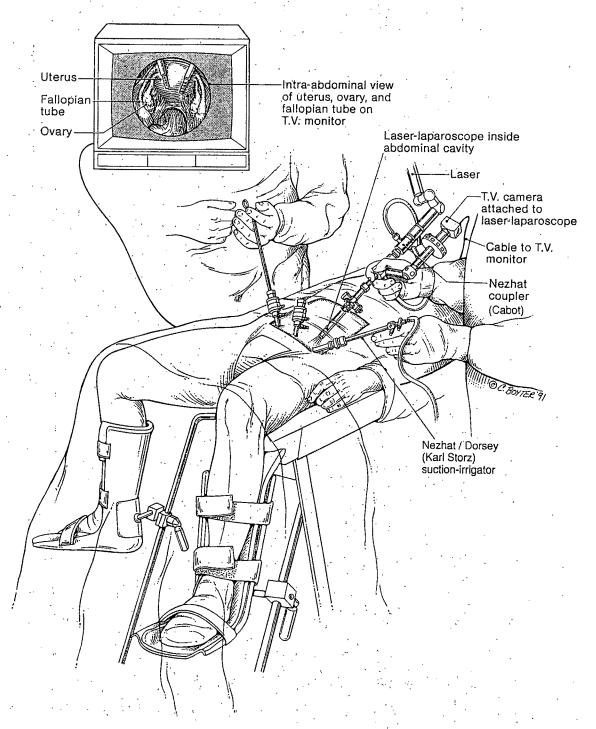


Fig. 1. Operating room setup.

The duration of oophorectomy was 22 to 55 minutes and was shortest in the patient requiring prophylactic oophorectomy, where no previous pelvic abnormality existed. Cases associated with myomectomy lasted 115 to 180 minutes, and laparoscopically assisted vaginal hysterectomy lasted 130 to 230 minutes.14, 15 While patients with laparoscopically assisted vaginal hysterectomy were discharged on the second or third postoperative day, all other patients were discharged within 24 hours, requiring an average hospital stay of 8.5 hours.

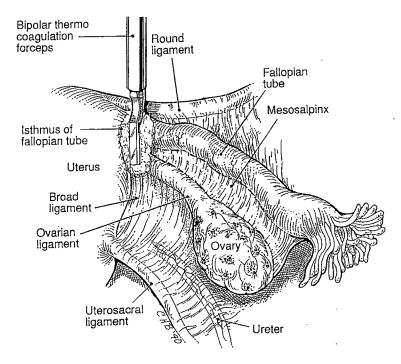


Fig. 2. Option 1: Coagulation of infundibulopelvic ligament adjacent to uterus.

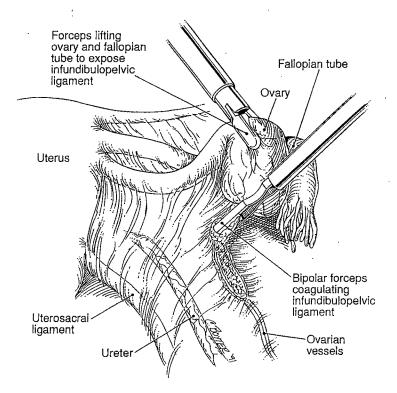


Fig. 3. Option 2: Coagulation if infundibulopelvic ligament distal to uterus.

Comment

Oophorectomy is frequently necessary in patients experiencing chronic pelvic pain because of adhesions or endometriosis unresponsive to conservative medical or surgical therapy. Large endometriomas and benign ovarian cysts can destroy normal ovarian tissue, and oophorectomy may be selected in these cases, at the judgment of the surgeon. In the past, laparotomy was

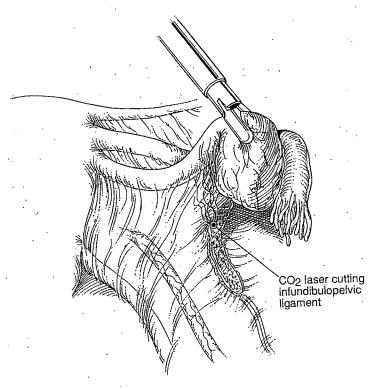


Fig. 4. Carbon dioxide laser used as a long knife through operative channel of laparoscope.

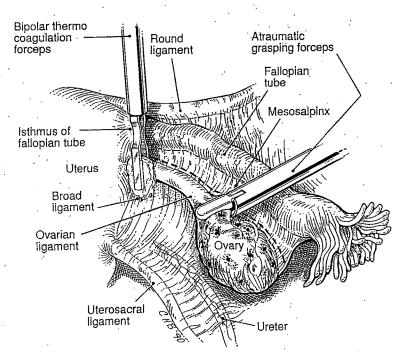


Fig. 5. Coagulation of ovarian ligament proximal to uterus.

the only surgical means to accomplish oophorectomy. Ovaries can now be removed at laparoscopy by experienced operative laparoscopists on an outpatient basis, resulting in shorter hospital stay, faster return to work, lower cost, and less adhesion formation. ^{16, 17}

Whereas laparoscopic oophorectomy has been reported with other techniques, videolaseroscopy offers some technical advantages to the operative laparoscopist. Perry and Upchurch, 18 using different instrumentations and techniques, performed oophorectomies in

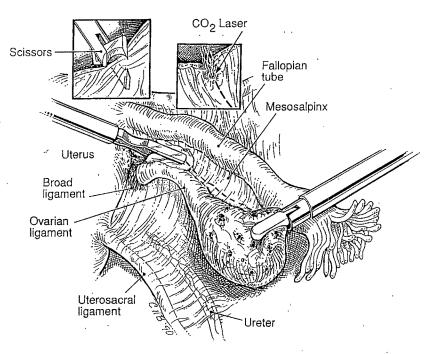


Fig. 6. Coagulation of ovarian ligament proximal to uterus.

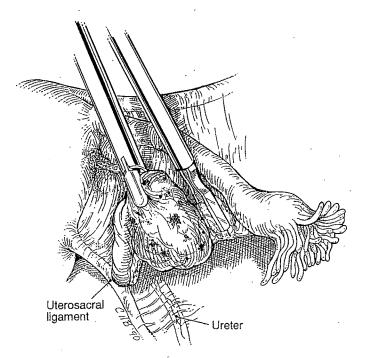


Fig. 7. Ovary has been freed from ureter and is under traction.

17 patients. One, with postoperative bleeding, required a laparotomy and transfusion. Six patients were excluded because of adhesions and an inability to visualize the ureter. In the majority of cases, pelvic adhesions must be lysed before the ovary can be mobilized, and ovarian anatomy is frequently distorted. Videolaseroscopy using the carbon dioxide laser and hydrodissec-

tion are an excellent combination for lysis of adhesions and entering the retroperitoneum¹⁹ to mobilize the ovary and remove it. In contrast, endoloop sutures cannot be applied in the presence of adhesions or distorted anatomy, and therefore additional instruments and techniques are required, thereby complicating the operating room setup. Furthermore, application of en-

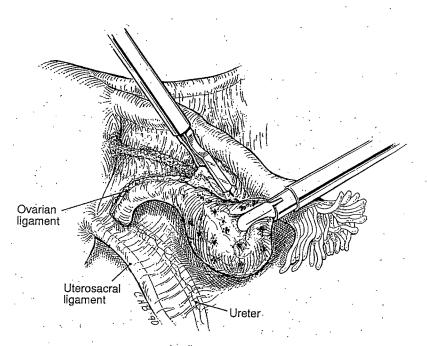


Fig. 8. Saving tube and removing ovary—position of ureter can be seen.

doloop sutures to large pedicles such as the mesovarium and infundibulopelvic ligament is awkward even in the absence of anatomic distortion. Once applied, the slip-knot can easily loosen under the tension of this large pedicle, increasing the risk of intraoperative hemorrhage and occasionally leaving a piece of ovary, causing ovarian remnant syndrome.

Complete desiccation of tissues with bipolar cautery, by means of generator amp meter, has been suggested before transection of pedicles.20 In our experience, however, desiccation to the point of blanching allows improved tissue healing without risk of hemorrhage. Overdesiccation (by allowing the amp meter to go to zero) creates friable tissue, which can actually predispose to intraoperative bleeding, and increased thermal damage that can theoretically produce postoperative adhesions. In addition, the pedals of the bipolar cautery device may stick to the tissue. Generators that provide a self-limiting bipolar desiccation mode minimize the chance for overdesiccation. Self-limiting bipolar cautery (Valley Lab Force series generators, Boulder, Colo.) provides controlled desiccation without charring the adjacent tissue. In this mode, the power peaks into a 100 Ω resistance instead of 300 to 500 Ω in typical generators. The power will then "roll off" at 1/resistance. This rapid but controlled "roll-off" provides the desired surgical effect without excess drying, blanching, or destruction of tissue.

Even after hemostasis has been achieved, in the presence of pneumoperitoneum, pedicles should be reinspected after evacuation of abdominal carbon dioxide. After intraabdominal pressure is lowered, ovarian pedicles have been noted to bleed again at termination of

the procedure. This maneuver offers one additional safeguard for complete hemostasis.²¹

Use of the carbon dioxide laser as the cutting instrument eliminates the tedious sequence of coagulating, removing the forceps, and introducing the scissors repetitively. In contrast, the laser remains available to the surgeon, always ready as a long knife, along the line of vision of the laparoscope, is operated by foot pedal instead of an additional hand, and functions to replace the scissors and, in many instances, the bipolar cautery as well. Finally, the posterior blade of the scissors becomes obscured by the tissue about to be cut, whereas the laser beam remains in view at all times, thereby eliminating any blind surgical manipulation.

In these 76 patients there were no major complications. Minor complications were associated with the laparoscopic procedure and not the oophorectomy. When there is no contraindication to laparoscopy, oophorectomy can be safely performed by an experienced operative laparoscopist at the time of diagnostic laparoscopy.

Laparoscopic oophorectomy appears to be a safe alternative to laparotomy, offering all the advantages of an outpatient surgical procedure. The techniques of videolaseroscopy simplify oophorectomy for the experienced operative laparoscopist.

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Inappropriate secretion of antidiuretic hormone in Sheehan's syndrome: A rare cause of postpartum hyponatremia

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A 27-year-old woman experienced hemorrhagic shock after delivery. One week later she was seen in an obtunded state of consciousness. The results of laboratory evaluation were consistent with the syndrome of inappropriate antidiuretic hormone secretion caused by hypopituitarism. Hydrocortisone rapidly corrected sodium levels. Syndrome of inappropriate secretion of antidiuretic hormone caused by Sheehan's syndrome should be considered in the differential diagnosis of postpartum hyponatremia. (Am J OBSTET GYNECOL 1991;165:1330-3.)

Key words: Antidiuretic hormone, hyponatremia, Sheehan's syndrome

Postpartum anterior pituitary necrosis (Sheehan's syndrome) infrequently causes hypopituitarism. Hyponatremia is a rare initial manifestation of postpartum pituitary insufficiency, particularly when it occurs within a few days of parturition. We report a case of

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Reprint requests: Chaim Putterman, MD, Department of Internal Medicine A, Hadassah University Hospital, Kiryat Hadassah, POB 12000, Jerusalem, Israel, 91120. 6/1/31190 A 27-year-old woman complained of headache and weakness. Seven days earlier she had been vaginally delivered of a healthy male infant. Immediately post partum she had an estimated 2 L uterine hemorrhage with shock. She was resuscitated with blood and colloids; hemorrhage was controlled with uterine massage, oxytocin, and ergotamine. One week after delivery she suddenly had paresthesias, slurred speech, headache, weakness, and somnolence. She also noted new diffi-

appropriate secretion of antidiuretic hormone.

Case report

A 27-year-old woman complained of headache and weakness. Seven days earlier she had been vaginally.

early Sheehan's syndrome initially seen as severe in-

culties with breast-feeding. Blood pressure was 120/80 mm Hg with no orthostatic change, and the heart rate was 80 beats/min. Eye grounds, visual fields, and the results of neurologic evaluation were normal. Axillary and pubic hair was present. The uterus was well contracted. The remainder of the results of the physical examination were within normal limits. The hematocrit was 28% and the hemoglobin was 9.9 gm/dl. The serum sodium level was 111 mmol/L, potassium 3.7 mmol/L, glucose 5.5 mmol/L, and urea 3.6 mmol/L. Urinalysis showed a sodium level of 116 mmol/L and a potassium level of 29 mmol/L. Baseline endocrine evaluation revealed markedly low levels of morning cortisol, 33.1 nmol/L (normal 110 to 520 nmol/L), and corticotropin < 1.1 pmol/L (normal 4 to 22 pmol/L). The triiodothyronine was 1.8 nmol/L (normal 1.2 to 3.4 nmol/L), thyroid-stimulating hormone 0.7 mU/L (normal 0.15 to 3.5 mU/L), prolactin 4.4 μ g/L (normal for a woman who is breast-feeding >50 μ g/L), growth hormone 1.2 $\mu g/L$ (normal < 5 $\mu g/L$), follicle-stimulating hormone 0.9 IU/L (normal 5 to 30 IU/L), and luteinizing hormone 21.4 IU/L (normal 5 to 20 IU/L). Therapy with intravenous isotonic saline solution and furosemide resulted in no significant change in blood sodium level. A 200 mg bolus of hydrocortisone was then administered intravenously, after which an additional 200 mg was continuously administered over 5 hours. Within hours, blood sodium levels increased to 123 mmol/L. Computed tomographic scan of the brain showed pituitary infarction with an increase in cerebrospinal fluid surrounding the gland (Fig. 1, A). When the patient received intravenous hydrocortisone therapy without fluid or salt restriction, normal sodium levels were attained and urine sodium level decreased to normal (Fig. 2). At 6 months' follow-up the patient was well and receiving maintenance therapy with hydrocortisone, levothyroxine, estrogen, and progesterone. Provocative pituitary testing confirmed panhypopituitarism. A follow-up computed tomographic scan of the brain demonstrated empty sella, with only remnants of the pituitary evident (Fig. 1, B). Complete results of the insulin-hypoglycemia, thyrotropin-releasing hormone and luteinizing hormone-releasing hormone tests are given in Fig. 3.

Comment

With the improvements in obstetric technique, the incidence of postpartum hemorrhage and subsequent pituitary insufficiency has appreciably decreased. Nevertheless, Sheehan's syndrome still causes hypopituitarism in women who bear children.

Chronic hyponatremia can infrequently appear in Sheehan's syndrome. However, hyponatremia as the initial manifestation of Sheehan's syndrome in the early postpartum period is extremely rare and has been reported only once. Sidorov and Mitnick¹ described an asymptomatic woman in whom a sodium level of 119 mmol/L was found 10 days after delivery. Evaluation confirmed panhypopituitarism, and the sodium level normalized with dexamethasone. However, this patient

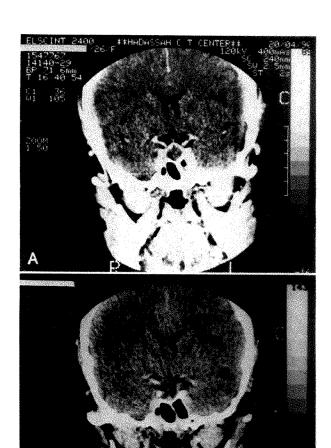


Fig. 1. A, Computed tomographic scan of brain demonstrating pituitary infarction with decrease in gland size. B, Repeat computed tomographic scan of brain shows almost total disappearance of pituitary tissue consistent with empty sella.

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was completely asymptomatic, and the sodium level was not life-threatening.

Several hypotheses have been advanced to explain the pathophysiologic mechanism of hyponatremia in Sheehan's syndrome. Hypothyroidism can cause decreased free water clearance and hyponatremia. However, the relative hypothyroidism in our patient was not sufficient to explain the marked degree of hyponatremia. Glucocorticoid deficiency can impair free water excretion through modulation of collecting duct permeability independent of vasopressin. Aldosterone secretion is generally regarded to be independent of pituitary regulation by corticotropin, although decreased aldosterone secretion may contribute to hyponatremia in some patients.

Glucocorticoid deficiency can cause hypovolemia and hypotension, leading to baroreceptor-mediated appropriate release of vasopressin. There was controversy as to whether the glucocorticoid deficiency of hypopituitarism can also cause inappropriate secretion of antidiuretic hormone. Oelkers2 recently found inappro-

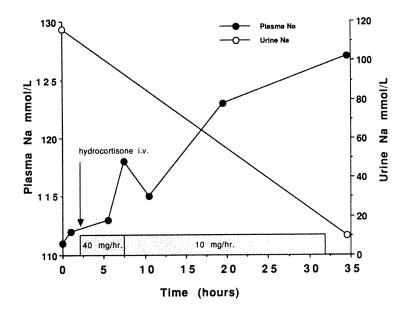


Fig. 2. Increase in blood sodium levels with concomitant decrease in urine sodium levels after hydrocortisone. *Arrow*, Start of hydrocortisone therapy (200 mg bolus); *shaded box*, duration of intravenous hydrocortisone therapy.

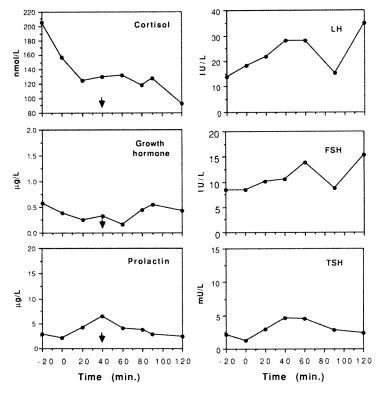


Fig. 3. Pituitary hormone response to combined pituitary function test. Thyrotropin-releasing hormone (400 μg) and gonadotropin-releasing hormone (100 μg) were administered at 0 minutes with 40 mU/kg/hr of insulin. *Arrows*, Time of nadir blood glucose level (2.3 mmol/L); *LH*, luteinizing hormone; *FSH*, follicle-stimulating hormone; *TSH*, thyroid-stimulating hormone.

priately high vasopressin levels relative to plasma osmolality in five women with chronic hypopituitarism and hyponatremia (two with Sheehan's syndrome). The relationship between antidiuretic hormone and osmo-

lality normalized with maintenance hydrocortisone therapy. Synthesis of vasopressin by the hypothalamus and its secretion by the posterior pituitary seems to be under inhibitory control by corticosteroids. Hypopi-

tuitarism is an important nonosmotic stimulus for antidiuretic hormone secretion and can cause severe inappropriate secretion of antidiuretic hormone.

In summary, we report a case of postpartum hypopituitarism that caused inappropriate secretion of antidiuretic hormone and hyponatremia. Replacement therapy with hydrocortisone increased sodium blood levels to normal. Even when appearing in the early postpartum period, severe hyponatremia can be the initial manifestation of Sheehan's syndrome.

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Congenital heart block: Successful prophylactic treatment with intravenous gamma globulin and corticosteroid therapy

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In mothers with anti-Ro-positive antibodies whose previous pregnancies have ended in deliveries of infants with congenital heart block, prophylactic therapeutic strategies are used to try to diminish the production and passage into fetal circulation of autoantibodies. Intravenous gamma globulin was given at 14 and 18 weeks' gestation and prednisone was given from 14 weeks' gestation to a woman with Sjögren's syndrome. The pregnancy ended with delivery of an infant without congenital heart block. (AM J OBSTET GYNECOL 1991;165:1333-4.)

Key words: Intravenous gamma globulin, congenital heart block, anti-Ro antibodies

Neonatal lupus syndrome and congenital heart block occur in infants whose mothers have antibodies to Ro (SS-A) and La (SS-B) autoantigens. The antibodies cross the placenta and may have a pathogenetic role in tissue damage in the fetus. Therefore therapeutic strategies such as plasmapheresis and corticosteroid therapy are used to diminish the autoantibody production and passage into the fetal circulation. The risk of congenital heart block is 1 in 20 pregnancies in which the mother has anti-Ro antibodies. If the mother has had an infant with congenital heart block, the risk in subsequent pregnancies is one in four. We report a high-risk pregnancy that was treated with intravenous gamma globulin and orally administered corticosteroids.

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Case report

The patient was a 38-year-old woman with primary Sjögren's syndrome and hypothyroidism that began in 1978. Her first pregnancy was normal, and she was delivered of a healthy infant in 1974. During her second pregnancy 14 years later Sjögren's syndrome was inactive. At 22 weeks' gestation fetal bradycardia (60 beats/min) was observed and a diagnosis of congenital heart block was made with ultrasonography. Double immunodiffusion determined that anti-Ro-antibody titers were elevated (1:8), but no anti-La antibodies were found. She was delivered of an infant whose heart was structurally normal except for congenital heart block. A pacemaker was inserted. One year later during her third pregnancy the anti-Ro-antibody titer was 1:1, but no anti-La antibodies were found. Intravenous gamma globulin (Sandoglobulin, Sandoz, Bern, Switzerland) was given at 14 and 18 weeks' gestation with a dose of 1 gm/kg during 16 hours of infusion. Prednisone was given at 14 weeks' gestation with an initial dose of 40 mg/day and tapered within 4 weeks to 10 mg/day until delivery. After the second infusion of gamma globulin, anti-Ro antibodies were not detectable by double-immunodiffusion technique (Fig. 1). Enzyme immunoassay was also used to detect nonprecipitating anti-Ro

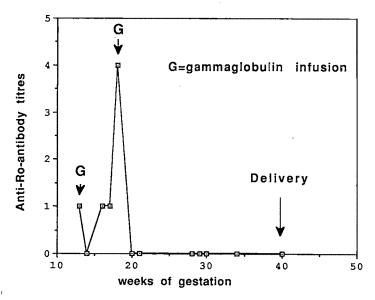


Fig. 1. Effect of intravenous gamma globulin on anti-Ro (SS-A) antibodies (1:1) measured with double-immunodiffusion technique.

antibodies. There was only a slight decrease in antibodies with solid-phase immunoassay. No signs of fetal bradycardia were observed, and a healthy child with normal heart function was born.

Comment

There are several mechanisms by which the combined corticosteroid and intravenous immunoglobulin therapy could be beneficial in an antibody-mediated disease. In addition to the simple antiinflammatory effect of corticosteroids and the dilution effect of intravenous immunoglobulin infusion, we can conjecture that there is a blockade of the transplacental pathway of antibodies.

Corticosteroids will also slowly diminish antibody productions. Jerne's theory of an idiotype-antiidiotype regulation of the immune system has gained strong experimental support. The antiidiotypes of normal human serum are able to interfere with autoantibodies present in a patient's sera. Such antiidiotypes are present in the intravenous immunoglobulin used in our case.

Thus it is possible that antiidiotypes are partly responsible for the decrease in the anti-Ro titer in the immunodiffusion test. This elimination may take place on the cellular (production) level or in the circulation (elimination). It is also possible that the antiidiotypes interfere in the immunodiffusion test used for the anti-Ro assay.

Interestingly, the therapy did not have a similar impact on the anti-Ro levels as measured with a solid-phase enzyme immunoassay. It is obvious that the immunodiffusion test and the solid-phase immunoassay detect different autoantibody populations. Most moth-

ers who have anti-Ro-positive antibodies give birth to normal children. Therefore it is possible that there is a pathogenetic subgroup of anti-Ro or other antibodies that are sensitive to immunomodulation by intravenous immunoglobulin.

The timing of therapeutic intervention is important in the prophylaxis of congenital heart block. There are no reports of successful outcome if the treatment has been started after fetal bradycardia is observed (usually >22 weeks' gestation). There are two reports in which a reduction of anti-Ro-antibody titers was achieved after steroid therapy and plasmapheresis and led to delivery of a healthy child. In these cases the treatment was given before 20 weeks' gestation.²

Our report of successful early prophylactic therapy is in agreement with these reports. Experimental prophylactic therapies should be restricted to high-risk pregnancies under research protocols with informed consent until controlled data are available. It is not clear that the good outcome of this case was related to the infusion of gamma globulin; it may be that the outcome was unrelated to the therapy. However, we think that intravenous immunoglobulin infusion is a good substitute for more laborious and hazardous techniques such as plasmapheresis. The immunoglobulin should be infused before the onset of bradycardia, which is an indicator of cardiac damage.

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Amniotic fluid contains tissue factor, a potent initiator of coagulation

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A primary clinical manifestation of amniotic fluid embolism is coagulopathy. Prior studies have identified a poorly characterized yet potent procoagulant property in amniotic fluid that increases with gestational age. One possible source of procoagulant activity is tissue factor, a primary biologic initiator of coagulation. We used sensitive immunoassays and functional assays to identify substantial quantities of tissue factor antigen and tissue factor—specific procoagulant activity in amniotic fluid, which increased with gestational age. Moreover, tissue factor accounted for virtually all of the coagulant potential of amniotic fluid. Amniotic tissue factor appeared intact and membrane bound and, when reconstituted into synthetic microvesicles of optimal phospholipid content, displayed nearly full activity. Calcium chelation and sonication experiments suggested that the presence of inhibitors and the physical configuration of membrane-bound tissue factor in amniotic fluid might explain the modest reduction in tissue factor procoagulant activity relative to total antigen levels observed in vivo. We postulate that the substantial quantities of functionally active tissue factor in amniotic fluid account for the coagulation changes accompanying amniotic fluid embolism and could indirectly contribute to the characteristic hemodynamic derangements of amniotic fluid embolism. (AM J OBSTET GYNECOL 1991;165:1335-41.)

Key words: Amniotic fluid embolism, tissue factor, lipoprotein-associated coagulation inhibitor

Amniotic fluid embolism is a rare but calamitous obstetric event, with maternal mortality rates exceeding 80%. The most common hemodynamic abnormality identified in affected patients is an elevation in pulmonary capillary wedge pressure accompanied by evidence of left ventricular failure. A potentially lethal clinical coagulopathy is present in 40% of cases; however, subtler coagulation abnormalities may be present in the remainder of patients.

While the mechanisms for the maternal hemodynamic derangements in amniotic fluid embolism remain obscure, the etiology of the concomitant coagulopathy is equally enigmatic. Amniotic fluid has been shown to have a direct factor X-activating property.² This property increases toward term, paralleling the

boplastin-like property is present when amniotic fluid is added to normal plasma, an effect that also increases with gestational age. These findings suggest that amniotic fluid may contain tissue factor or other components of the extrinsic pathway of coagulation.

development of fetal pulmonary maturity.3 A throm-

Coagulation has traditionally been studied in a glass tube under static conditions. However, such investigations focused on circulating intrinsic pathway components and not cell-mediated coagulation. The latter is initiated by tissue factor, and it is this extrinsic pathway that maintains hemostasis in vivo.⁵ Tissue factor has an apparent molecular weight of 40,000 to 46,000.⁶ It is a true cofactor and does not require proteolysis for activation but must be membrane bound for activity.^{5,7}

Disruption of vascular integrity is required for tissue factor—mediated hemostasis, because tissue factor is sequestered in the subendothelium and adventitia and does not circulate in plasma. Once exposed to blood, tissue factor binds to factor VII, which, although a zymogen, is partially active. Because cleavage of factor VII results in a 120-fold increase in this activity, the designation factor VII(a) is used to indicate that both zymogen and enzyme are active. The affinity of tissue factor for factor VIIa is increased in the presence of acidic membrane phospholipids. The complex of tissue factor—factor VII(a) can activate either factor X or

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IX and thus trigger clotting. Once tissue factor is exposed to blood, its potent procoagulant effects are eventually inhibited by lipoprotein-associated coagulation inhibitor, a 38,000 molecular weight protein that forms a calcium-dependent quaternary complex with tissue factor, factor VIIa, and factor Xa. 10-12

The purpose of this investigation was to determine whether tissue factor and other extrinsic pathway components are present in the amniotic fluid and therefore contribute to the pathogenesis of amniotic fluid embolism.

Material and methods

After we received informed consent from the patients, amniotic fluid specimens were obtained from amniocenteses for fetal pulmonary maturity (37 to 39 weeks' gestation) and karyotype (16 to 18 weeks) studies. Sample volumes were 5 and 2 ml, respectively. All specimens with meconium or gross or microscopic evidence of red blood cell contamination were discarded. Samples obtained from pregnancies complicated by maternal infection, abruptio placentae, multiple gestations, and fetal anomalies were excluded. All amniotic fluid specimens were maintained at -80° C until used. For the purpose of analysis specimens within 2 weeks of gestation were subsequently pooled in volumes of 20 to 30 ml. These pooled specimens (n = 7 for each trimester) were then aliquoted into a whole amniotic fluid sample, a cell-free, low-speed (1000g for 15 minutes at 4° C) centrifugation supernatant containing membrane microvesicles, and a corresponding lowspeed, cell-enriched pellet.

Tissue factor enzyme-linked immunosorbent assay. Membrane-bound tissue factor was solubilized by the addition of 0.1% Triton X-100 and measured with a sensitive enzyme-linked immunosorbent assay (ELISA).13 This ELISA used a well-characterized murine monoclonal antibody against human tissue factor in the solid phase¹⁴ and a polyclonal anti-tissue factor antibody to amplify the murine monoclonal antibodybound tissue factor signal. The polyclonal antibody was raised as described13 and affinity purified by protein A-Sepharose and a human tissue factor-affi-Gel column (Bio-Rad, Hercules, Calif.). Goat antirabbit immunoglobulin G conjugated to horseradish peroxidase (Boehringer-Mannheim, Indianapolis) and the substrate orthophenyldiamine were used to visualize the complex. After acid quenching, the optical density at 490 nm was read with an automated microplate reader (Molecular Devices, Menlo Park, Calif.). Concentrations of amniotic fluid tissue factor were derived in triplicate from a standard curve with the SOFTMAX computer program (Molecular Devices). The linear

range of the standard curve run with each assay was 0 to 155 μ g/ml (r > 0.99). Intraassay variations were <5%.

Tissue factor functional assay. A modification 15 of the two-stage tissue factor clotting assay of Nemerson¹⁶ was used to measure concentrations of functionally active human tissue factor in amniotic fluid specimens. Reagents included human factor VIIa and factor X prepared by barium citrate adsorption followed by ionexchange chromatography and molecular size separation with an ultragel AcA-44 column (Bio-Rad), as described. 17, 18 The linear range of the assay's standard curve was 5 to 200 pg/ml. Amniotic factors VIIa and Xa did not contribute to spurious estimates of tissue factor concentration because repetition of each assay in the absence of added factor VIIa or X greatly prolonged the clotting time. To further confirm that tissue factor was primarily responsible for amniotic fluidinduced clotting, parallel samples of amniotic fluid were preabsorbed with molar excess anti-tissue factor murine monoclonal antibody before assay.

Immunoblot and tissue factor-membrane reconstitution studies. To determine if amniotic fluid tissue factor was degraded or synthesized in a truncated, potentially inactive form, solubilized tissue factor derived from whole amniotic fluid was extracted by immunoaffinity purification, and its electrophoretic size was evaluated by immunoblot analysis, in comparison with immunopurified human brain tissue factor, as described by Bach et al.19 In addition, a known amount of immunopurified amniotic fluid tissue factor was suspended in 0.1% Triton in 0.1 mol/L sodium chloride and 0.05 mol/L Tris buffer, pH 7.5, precipitated in cold acetone (0° C), and resuspended in 375 mmol/L of the nonionic dialyzable detergent octyl-β-D-glucopyanoside (Sigma Chemical Co., St. Louis) with phosphatidylserine and phosphatidylcholine. Subsequent dialysis of octyl-\beta-p-glucopyanoside resulted in formation of reconstituted microvesicles with a tissue factor/phospholipid ratio of 1:100,000 and a phosphatidylserine/phosphatidylcholine ratio of 30:70, optimal for tissue factor procoagulant activity.7, 9 These reconstituted microvesicles were then assayed for both tissue factor antigen and activity.

Sedimentation analysis of amniotic tissue factor. To ascertain why amniotic fluid tissue factor procoagulant activity was reduced relative to total tissue factor antigen levels, amniotic fluid cell-free, low-speed supernatant fractions from the second and third trimesters were subjected to either (1) calcium chelation with 20 mmol/L ethylenediaminetetraacetic acid for 30 minutes at 20° C to disrupt putative calcium-dependent tissue factor—inhibitor complexes, (2) probe sonication

for 30 seconds three times at 0° C to disrupt possible multilayered membrane macroaggregates that could impede tissue factor-factor VII(a) binding, (3) both of these, or (4) neither of these. Ultracentrifugation at 143,000 g for 16 hours at 4° C was then used to separate sedimented membrane-bound tissue factor from free tissue factor, coagulation factors, and inhibitors. This high-speed pellet and supernatant were then assayed for tissue factor antigen and activity.

Assays for lipoprotein-associated coagulation inhibitor. To assess specific sources of tissue factor inhibition in amniotic fluid, pooled whole amniotic fluid and cell-free, low-speed supernatant specimens from the second and third trimesters were assayed for lipoprotein-associated coagulation inhibitor concentrations by ELISA and a functional tissue factor inhibition assay as described.20, 21

Relipidation experiments. To determine if variations in amniotic fluid membrane phospholipid constituents contributed to the observed disparities between tissue factor antigen concentrations and activity, the cell-free, low-speed supernatant and high-speed pellet from both second- and third-trimester amniotic fluid specimens were subjected to either (1) membrane disruption with 375 mmol/L octyl-β-D-glucopyanoside alone or (2) 375 mmol/L octyl-β-D-glucopyanoside plus phosphatidylserine and phosphatidylcholine to produce tissue factor/phosphatidylserine/phosphatidylcholine microvesicles of optimal coagulation potential.19 After incubation at 37° C for 2 hours, samples were exhaustively dialyzed in 0.05 mol/L Tris buffer, pH 7.5, and assayed for tissue factor antigen and activity

Coagulation assays for factors IIa, Xa, and VII(a). Amniotic fluid factor IIa concentrations were assessed by amidolytic assay with the thrombin-specific substrate H-D-hexahydrotyrosyl-L-alanyl-L-arginine-pnitroanilide22 according to the manufacturer's specifications (Sp-TH, American Diagnostica, Inc., New York). Absorbance readings, corrected for the intrinsic absorbance of amniotic fluid at 405 nm, were obtained after acid quenching, and amniotic fluid factor Ha levels were derived from a factor IIa (Sigma) standard curve. Amniotic fluid factor Xa concentrations were assessed by a similar amidolytic assay with the use of a specific factor Xa chromogenic substrate, MeO-CO-D-Gly-Arg-nitroanilide (Sp-Xa, American Diagnostica), and purified factor Xa18 as a standard. The percentage of amniotic fluid factor X existing in the zymogen versus active form was assessed by preincubation of samples with a direct, calcium-independent activator of factor X, Russell's viper venom (Sigma).

Amniotic factor VII(a) levels were measured by a

modified prothrombin time with the use of factor VII(a)-deficient plasma, according to the manufacturer's specifications (Baxter Healthcare Corp., Miami). Immunopurified human factor VIIa, prepared as described,17 was used as a standard, and human recombinant tissue factor (Genetech, South San Francisco) was used as a thromboplastin source at 50 ng/ml. The specificity of this assay for VII(a) was confirmed by prior incubation of parallel amniotic fluid specimens with selective covalent inhibitors of factor Xa and IIa, Phe-Pro-Arg-chloromethylketone (Calbiochem, San Diego) (1 µmol/L) and dansyl-Glu-Gly-Arg-chloromethylketone (Calbiochem) (10 µmol/L) (Guha A, Nemerson Y. Unpublished observations) respectively. The proportion of amniotic fluid factor VII existing in the zymogen versus active state was assessed by prior incubation of amniotic fluid with the specific covalent inhibitor of factor VIIa, Phe-Phe-Arg-chloromethylketone (Calbiochem) (10 μmol/L),23 followed by exhaustive dialysis of excess inhibitor.

Protein assay. Protein concentrations of amniotic fluid specimens were measured by a modified Bradford assay (Bio-Rad, Richmond, Calif.).

Results

Tissue factor ELISA and functional assay. Concentrations of tissue factor per milligram of amniotic fluid protein in whole amniotic fluid, its derivative cell-free supernatant, and cell-enriched pellet are presented in Table I. Term amniotic fluid specimens demonstrated higher tissue factor concentrations whether measured by ELISA or functional assay compared with those of second-trimester specimens. Whereas gestational age differences in tissue factor antigen levels were a consequence of the higher amniotic fluid protein concentrations in the second trimester compared with term specimens (8.8 \pm 2.4 vs 4.8 \pm 1.6 mg/ml, p = 0.002), functional tissue factor concentrations measured in nanograms per milliliter of fluid were significantly increased at term compared with those of the second trimester $(0.89 \pm 0.19 \text{ vs } 0.49 \pm 0.24 \text{ ng/ml}, p =$ 0.01). Moreover, the ratio of tissue factor concentrations measured by functional assay versus ELISA were consistently greater in term compared with secondtrimester specimens (Table II). Preadsorption of amniotic fluid with excess anti-tissue factor murine monoclonal antibody virtually eliminated amniotic fluidinduced clotting in the functional assay, suggesting that tissue factor was responsible for nearly all the procoagulant activity of amniotic fluid.

Immunoblot and tissue factor-membrane reconstitution studies. Immunoblot analysis of immunoaffinity purified tissue factor from second- and third-trimester

Table I. Amniotic fluid tissue factor concentrations (in picograms per milligram of total protein)

	Sec	Second trimester		Third trimester	
Source and Assay	Mean	95% Confidence interval	Mean	95% Confidence interval	p Value*
Whole amniotic fluid (ELISA)	654	419-890	1118	699-1537	0.038
Cell-free, low-speed supernatant (ELISA)	579	,363-795	870	628-1112	0.049
Cell pellet from low-speed centrifugation (ELISA)	1044	574-1514	2534	1361-3706	0.02
Whole amniotic fluid (functional assay)	59	27-92	203	134-272	0.001
Cell-free, low-speed superna- tant (functional assay)	50	22-78	. 148	101-195	0.001
Cell pellet from low-speed centrifugation (functional assay)	67	28-105	314	178-450	0.004

^{*}Student t test.

Table II. Amniotic fluid tissue factor: ratio of concentrations determined by functional assay versus ELISA

•	Sec	Second trimester		Third trimester	
Source	Mean	95% Confidence interval	Mean	95% Confidence interval	p Value*
Whole amniotic fluid	0.093	0.046-0.140	0.188	0.142-0.234	0.004
LSS	0.086	0.058-0.113	0.180	0.125-0.234	0.004
LSP	0.064	0.044-0.083	0.142	0.071-0.213	0.04

^{*}Student t test.

samples consistently demonstrated two discrete, intact forms of tissue factor, as previously described ¹⁵ (Fig. 1). The higher-molecular-weight (46,000) form of tissue factor predominated in amniotic fluid, whereas human brain tissue factor displayed its major band at 40,000. Reconstitution of immunopurified amniotic fluid tissue factor into synthetic microvesicles of optimal phospholipid content restored nearly all the potential tissue factor activity (function/antigen = 0.91). Thus amniotic fluid tissue factor exists in an intact and potentially fully active form.

Sedimentation analysis of amniotic tissue factor. All amniotic fluid tissue factor appears to be membrane bound, because the ultracentrifuged supernatants contained negligible quantities of tissue factor antigen and activity. Both calcium chelation and probe sonication resulted in substantial increases in tissue factor activity relative to a given amount of antigen in the high-speed pellets. Table III presents the findings of a representative experiment, which suggests that both the physical configuration of tissue factor within a membrane complex and calcium-dependent tissue factor inhibitors in amniotic fluid may contribute to the observed relative reduction in tissue factor activity.

Assays for lipoprotein-associated coagulation inhibitor. Concentrations of lipoprotein-associated coagulation inhibitor antigen were 26.0 and 28.6 ng/ml in second-trimester whole amniotic fluid and cell-free, low-speed supernatant samples, respectively. Comparable values from a term specimen were 19.8 and 23.9 ng/ml, respectively. However, lipoprotein-associated coagulation inhibitor functional activity could not be identified in any specimen in spite of its nearly fourfold molar excess when compared with tissue factor in these specimens.

Relipidation experiments. No significant increase in tissue factor functional activity resulted from phospholipid reconstitution of native amniotic fluid microvesicular membranes at a phosphatidylserine/phosphatidylcholine ratio optimal for tissue factor functional activity. Thus amniotic fluid membrane phospholipid constituents do not appear to contribute to decreased activity within and between trimesters.

Coagulation assays for factors IIa, Xa, and VII(a). Low levels of factors Xa, VII(a), and IIa were identified in amniotic fluid (Table IV). Amniotic factor IIa levels appeared to be increased at term. Preincubation of amniotic fluid with Russell's viper venom had no effect on

107k

Fig. 1. Western blot of immunopurified tissue factor from pooled second-trimester amniotic fluid specimens, performed as described by Bach et al. 19 From left to right: lane A, Human brain tissue factor (50 ng); lane B, molecular weight standards (BRL Life Technologies, Inc., Gaithersburg, Md.); lane C; tissue factor (11 ng) from fraction 2 of affinity column elution. Note that tissue factor derived from both human brain and amniotic fluid appears intact. However, major electrophoretic band for tissue factor derived from human brain has apparent molecular weight of 40,000 (small arrow), whereas major band of amniotic fluid-derived tissue factor has molecular weight of 46,000 (large arrow). Identical immunoblot findings were obtained for amniotic fluid tissue factor derived from term specimens. (46, 46,000-Molecular-weight standard; 107k, 107,000-molecular-weight standard.)

Table III. Amniotic fluid tissue factor concentrations measured by ELISA and functional assay after calcium chelation with or without sonication and ultracentrifugation (picograms per milligram total protein)

		Second trimester		Third trimester		
Source	ELISA	Functional assay	· Ratio	ELISA	Functional assay	Ratio
HSP	563	149 .	0.26	934	115	0.12
HSP + E	392	201	0.51	1246	474	0.33
HSP + S	376	188	0.50	960 ·	181	$0.19 \\ 0.50$
HSP + E + S	440	220	0.50	1291	651	

HSP, Membrane-enriched pellet derived from ultracentrifugation of cell-free amniotic fluid supernatant containing membrane microvesicles (see Material and methods for details). HSP + E, HSP in which original amniotic fluid cell-free supernatant is preincubated with 20 mmol/L ethylenediaminetetraacetate. HSP + S, HSP in which original amniotic fluid cell-free supernatant is subjected to probe sonication. HSP + E + S, HSP in which original amniotic fluid cell-free supernatant is preincubated with 20 mmol/L ethylenediaminetetraacetate and sonicated.

factor Xa measurements, confirming that the active form of factor Xa predominates in amniotic fluid. Preincubation with a factor VIIa-specific inhibitor indicated that the zymogen, factor VII, is the predominant moiety in second-trimester whole amniotic fluid, whereas the active moiety, factor VIIa, predominates at term.

Comment

This study has systematically evaluated tissue factor and other extrinsic pathway components and potential inhibitors in amniotic fluid. We have found substantial tissue factor procoagulant activity in amniotic fluid, although it is relatively reduced in comparison with the amount of antigen. We have been able to account for

Table IV. Amniotic fluid factor Xa, VII(a), and IIa concentrations (in nanomoles per liter)

Factor and source	Second trimester (means ± SD)	Third trimester (means ± SD)	p Value*
Xa			
White amniotic fluid	0.09 ± 0.10	0.56 ± 0.12	0.025
Cell-free, low-speed supernatant	0.07 ± 0.06	0.13 ± 0.18	0.80
Cell pellet from low-speed centrifugation	31.4 ± 7.0 .	5.79 ± 7.03	0.07
VII(a)			
White amniotic fluid	0.22 ± 0.06	0.25 ± 0.09	0.48
Cell-free, low-speed supernatant	0.27 ± 0.04	0.39 ± 0.30	0.48
Cell pellet from low-speed centrifugation	0.56 ± 0.38	0.65 ± 0.77	1.0
IIa			
White amniotic fluid	0.05 ± 0.05	3.67 ± 4.9	0.009
Cell-free, low-speed supernatant	0.05 ± 0.05	0.91 ± 0.95	0.03
Cell pellet from low-speed centrifugation	2.77 ± 5.5	33.49 ± 34.4	0.009

^{*}Mann-Whitney two-sample test.

prior observations that whole amniotic fluid-induced coagulation activity increases with gestational age by identifying higher amniotic fluid tissue factor procoagulant activity at term compared with the second trimester. We have also noted that amniotic fluid tissue factor exists in an intact, membrane-bound state without evidence of degraded or truncated forms. The predominance of the higher-molecular-weight form for tissue factor derived from amniotic fluid compared with brain-derived tissue factor may reflect different glycosylation patterns. However, such differences are unlikely to effect coagulation potential, because glycosylation does not alter tissue factor procoagulant activity.7 Similarly, the phospholipid content of membranes does not appear to account for the decrease in procoagulant activity relative to total antigen levels.

In contrast, the physical conformation of membrane-bound tissue factor in amniotic fluid may reduce functional activity. Membrane macroaggregates could prevent access by bound tissue factor to free factor VII(a). Calcium-dependent inhibitors also may reduce activity. An immunoassay for a plasma tissue factor inhibitor, lipoprotein-associated coagulation inhibitor, demonstrated high antigen levels; however, this lipoprotein-associated coagulation inhibitor appeared inactive. Perhaps amniotic fluid lipoprotein-associated coagulation inhibitor is degraded or subject to a unique inhibitor. Alternatively, trophoblast-derived, calcium-dependent tissue factor inhibitors, such as placental anticoagulant protein,²⁴ may be present in amniotic fluid.

The source of tissue factor in amniotic fluid remains unknown. Cultured WISH amniotic cells synthesize tis-

sue factor.⁷ Sloughed fetal skin is a likely source, because the outer epidermis is rich in tissue factor.²⁵ Respiratory tract, gastrointestinal, and genitourinary mucosal epithelia also contain tissue factor and could contribute to the amniotic fluid tissue factor pool.

The presence of substantial tissue factor—specific procoagulant activity in amniotic fluid and the inhibition of amniotic fluid procoagulant activity by anti—tissue-factor antibodies suggest that tissue factor accounts for the bulk of the potent procoagulant properties of amniotic fluid. Moreover the markedly low concentrations of factors IIa, VIIa, and Xa measured in amniotic fluid (Table IV) stand in contrast to the mean maternal plasma concentrations of their zymogens at term (2300, 34.2, and 254 nmol/L, respectively). This suggests that amniotic fluid—derived activated coagulation factors probably contribute minimally to the coagulation abnormalities accompanying amniotic fluid embolism.

We speculate that the deportation of sufficient quantities of whole amniotic fluid—derived tissue factor into the pulmonary microvasculature will engender a number of disparate effects. Local factor IIa (thrombin) generation could cause vasoconstriction and microvascular thromboses.²⁸ Thrombin also can stimulate vascular endothelin secretion in cultured bovine endothelial cells.²⁹ Endothelin has been shown to increase left atrial pressure and depress cardiac output in a canine model,³⁰ effects also observed in humans with amniotic fluid embolism. Moreover, endothelin receptors have been detected in human coronary arteries.³¹ Therefore both the hemodynamic and coagulation effects of am-

niotic fluid embolism could theoretically result from deportation of active tissue factor into the pulmonary microcirculation.

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Laser vaporization of grade 3 vaginal intraepithelial neoplasia

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Between March 1, 1984, and May 23, 1990, 26 patients underwent laser vaporization for grade 3 vaginal intraepithelial neoplasia. Twenty of these patients had prior hysterectomy, 12 of them because of cervical neoplasia. Ten patients had undergone prior treatment for vaginal intraepithelial neoplasia. Eleven (42%) developed recurrence of vaginal neoplasia with a mean time to recurrence of 22 weeks. Three of the 11 patients had invasive cancer at the time of recurrence. One patient had invasive cancer on biopsy at the time of laser vaporization and subsequently underwent radiation therapy. The remaining 14 patients remain alive with no evidence of recurrent disease at a mean follow-up interval of 117 weeks. In our hands, laser vaporization did not appear to be efficacious treatment for grade 3 vaginal intraepithelial neoplasia, especially when diagnosed in the region of a vaginal cuff scar. (AM J OBSTET GYNECOL 1991;165:1342-4.)

Key words: Laser, vaginal neoplasia

Vaginal intraepithelial neoplasia accounts for approximately 0.4% of lower genital tract intraepithelial neoplasia.1 Identifiable risk factors for this condition include cervical intraepithelial neoplasia,2 previous pelvic radiotherapy,3 human papillomavirus infection,4 and immunosuppression.2-5 The invasive potential of vaginal intraepithelial neoplasia appears to be similar to that of cervical intraepithelial neoplasia.2,6 The process most commonly occurs in the upper third of the vagina and is frequently multifocal.7-11 Vaginal intraepithelial neoplasia is usually diagnosed during investigation of an abnormal Papanicolaou smear; it is not grossly visible and does not usually produce symptoms. The diagnosis is established by colposcopy with directed biopsy. A wide variety of treatments have been used, most commonly consisting of local excision, partial or total vaginectomy, radiotherapy, laser vaporization, or topical 5-fluorouracil.12 Each of these treatment methods have different advantages and disadvantages, and none has proved superior efficacy over the others. This is a report of our experience with the use of laser vaporization for grade 3 vaginal intraepithelial neoplasia.

Material and methods

The charts of all patients with grade 3 vaginal intraepithelial neoplasia managed by our division between Jan. 1, 1984, and June 1, 1990, were reviewed retro-

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It was not possible to accurately define how patients were selected for laser vaporization over some other form of treatment. All patients underwent colposcopy of the entire lower genital tract. A directed biopsy of visualized lesions was performed, with separate biopsies performed of areas with varying appearance. Large and small swabs were used to aid visualization. Skin hooks were not used. In general, our division's criteria for laser vaporization of grade 3 vaginal intraepithelial neoplasia include a completely visualized lesion that has been sampled as indicated with no suspicion for invasive cancer and that cannot be managed by a small local excision. All laser procedures were performed by an obstetrics and gynecology resident under the direct supervision of one of the five attending physicians. All procedures were performed in the operating room with the patient under general or regional anesthesia. All procedures were performed with colposcopic guidance and by a Sharpland 1060 carbon dioxide laser at a power density of 500 to 1000 W/cm². Lesions were vaporized to a depth of 1 to 2 mm, including at least a 1 cm margin of surrounding normal-appearing mucosa. All cases were managed on an outpatient basis unless concomitant procedures required an additional stay. Patients were examined at 2 weeks, 8 weeks, and then every 2 to 3 months after surgery for the first 2 years and every 6 months thereafter, unless there was recurrence of disease. All new lesions diagnosed in the follow-up period were, for purposes of this report, considered recurrences.

spectively. A total of 41 patients had been treated, 26

either primarily or secondarily with laser vaporization.

Results

The mean age of the 26 study patients was 57 years (range, 19 to 80 years). Twenty (77%) patients had

Table I. Analysis of recurrence

	Recurrence	No recurrence
Total No.	11 (42%)	15
Prior hysterectomy	11/11	9/15
Vaginal cuff involvement	10/11	5/15
Prior vaginal intraepithelial neoplasia/human papillomavirus treatment	5/11	5/15
No prior vaginal intraepithelial neoplasia/human papillomavirus treatment	6/11	10/15
Immunosuppression	2/11	0/15
Invasive cancer	3/11	NA
Prior cervical cancer	4/11	2/15
Prior radiotherapy	1/11	3/15

NA. Not available.

*All three patients with invasive cancer at the time of recurrence had undergone radical hysterectomy because of invasive squamous cell carcinoma of the cervix.

undergone hysterectomy. Eight of these were because of benign disease and 12 were because of squamous neoplasia. Four patients had undergone pelvic radiotherapy because of malignancy. Three of these were squamous cervical cancer and one was endometrial cancer. Ten patients (38%) had undergone therapy for vaginal intraepithelial neoplasia or vaginal human papillomavirus. These treatments included one laser vaporization in two patients, two laser vaporizations in one patient, upper vaginectomy in one patient, cautery in one patient, cryotherapy in one patient, 5fluorouracil cream in two patients, and multiple treatments in two patients.

The vaginal intraepithelial neoplasia was located in the upper third of the vagina in 15 of the patients, in the upper half of the vagina in three, in the anterior midvagina in one, and at multifocal sites in the remaining seven. Seven of the patients underwent additional procedures at the time of laser vaporization. Three of these were because of cervical intraepithelial neoplasia, three because of vulvar intraepithelial neoplasia, and one because of both cervical and vulvar intraepithelial neoplasia. The mean operative time was 42 minutes, with a range of 15 to 60 minutes. The estimated blood loss was reported as scant to <5 ml in all patients. There were no reported intraoperative complications and two reported postoperative complications. One patient had difficulty with diabetic control in the immediate postoperative period and another who was severely immunosuppressed had problems with healing, with essentially minimal healing at the time of a small recurrence 8 weeks after operation.

Recurrent vaginal neoplasia developed in 11 (42%) of the patients (Table I). The mean time to recurrence was 22 weeks, with a range of 8 to 78 weeks. Two of the recurrences were diagnosed at 64 and 78 weeks and may have represented new lesions. All 11 patients had undergone hysterectomy. In 10 of the 11 patients both initial and recurrent disease were at the apex of the vagina around the vaginal cuff scar. Four of the 11 patients had significant risk factors for recurrence, including two severely immunosuppressed patients, one patient who had undergone pelvic radiotherapy, and one patient who had experienced multiple treatment failures for vaginal intraepithelial neoplasia. Three of the 11 patients had invasive cancer at the time of the recurrence; all had been treated for invasive carcinoma of the cervix and all underwent radiotherapy. One patient eventually died as a result of the cancer. In two of the three patients it was thought that the invasive cancer had developed from epithelium that was buried within the vaginal cuff scar; these cases have been reported previously.18 One patient had superficially invasive cancer on a biopsy specimen obtained just before laser vaporization; she received radiotherapy after operation and was excluded from follow-up analysis. Invasive cancer at the time of recurrence was diagnosed in all three patients by pathologic examination of an upper vaginectomy specimen. Four other patients with recurrence underwent upper vaginectomy; one was treated with 5-fluorouracil cream, and three received multiple types of treatments. Fourteen patients remain alive with no evidence of recurrent disease at a mean follow-up interval of 117 weeks (range, 46 to 240 weeks). Follow-up information regarding anatomy and sexual function was inadequate for reporting.

Comment

A wide variety of methods have been used to treat vaginal intraepithelial neoplasia, including partial to total vaginal excision, radiotherapy, laser vaporization, and topical 5-fluorouracil.2,7,9,12-24 Good results generally have been obtained with these methods, although the results have been more variable with laser vaporization.12, 14-17, 19-24 Laser vaporization for vaginal intraepithelial neoplasia has been variously reported to have a 50% to 100% success rate. Some of these studies have included patients who have undergone two laser vaporizations. Partial or total vaginectomy has been considered by many to be the treatment of choice, mainly because it excises the abnormal tissue and provides a specimen for complete histologic review. 18, 18, 19 However, several advantages of laser vaporization and 5-fluorouracil have been cited, including reduced operative morbidity, less operative time, and improved subsequent sexual function. 14-18, 20-23 Laser vaporization or 5-fluorouracil also may be preferable in cases of previous irradiation, because of the excessive morbidity associated with vaginectomy. Patients who have a tendency toward recurrence of vaginal intraepithelial neoplasia also may benefit from these treatments and the reduced amount of shortening and scarring that may result from multiple applications of other forms of treatment (i.e., excision).

Before treatment with laser vaporization is considered, an accurate appraisal of the extent and severity of the disease must be made. This is facilitated by using colposcopy, Lugol's iodine, palpation, and multiple biopsies. Laser vaporization should not be performed unless the full extent of the abnormal vaginal epithelium can be visualized, there is no suspicion of an invasive process, and there is no gross scarring or distortion of the posthysterectomy vaginal cuff. The last point has been made by several authors, including a prior study from this institution, and a few isolated case reports of vaginal intraepithelial neoplasia and invasive cancer arising from such vaginal cuffs have been published. 13-21 The results of this study underline this problem.

Vaginal intraepithelial neoplasia in the upper vagina of women who have undergone hysterectomy probably should be excised along with the vaginal cuff scar. This seems especially important for patients in whom hysterectomy was performed because of cervical cancer or intraepithelial neoplasia, in whom the vaginal cuff is prominent or distorted, or who have had treatment failure with laser vaporization or topical 5-fluorouracil. On the basis of the results of this study, we believe that laser vaporization should not be used for the treatment of grade 3 vaginal intraepithelial neoplasia diagnosed in the region of a vaginal cuff scar. Laser vaporization certainly could be combined with an excision of the cuff scar area.

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On reducing the frequency of severe abruptio placentae

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At Parkland Memorial Hospital the frequency of abruptio placentae so severe as to kill the fetus has decreased from 1 in 420 deliveries during 1956 through 1969 to 1 in 830 during 1974 through 1989. Major factors in this reduction were elimination of very high parity and a marked increase in the percentage of Latin American women, in whom the risk was 1 in 1473 deliveries compared with 1 in 595 for black women and 1 in 876 for white women. Abdominal trauma was encountered rarely, as was fetoplacental-to-maternal hemorrhage sufficient to impair fetal perfusion seriously. Abnormal development of Müllerian ducts and uterine myomas were encountered rarely. Neither red blood cell macrocytosis characteristic of folate deficiency nor iron deficiency could be implicated in the genesis of severe abruptio placentae. Abruptio placentae recurred in 12% of subsequent pregnancies and proved fatal to the fetus in 7%, unchanged from our earlier experience. (AM J OBSTET GYNECOL 1991;165:1345-51.)

Key terms: Severe abruptio placentae, recurring abruptio placentae, high parity and abruptio placentae, race and abruptio placentae

Severe abruptio placentae, defined as premature placental separation of such magnitude as to kill the fetus, persists as an important cause of serious maternal morbidity and even mortality and a prominent cause of fetal death. Previously published studies from Parkland Hospital on 201 cases managed during 1956 through 1969 identified high parity, hypertension, and recurrent abruptio placentae to be much more common in pregnancies so complicated when compared with the general obstetric population.1 It was commented then that, "Hopefully, identification of factors of etiologic importance in the genesis of severe placental abruption will lead to a reduction in the frequency of this serious lesion." Indeed, as described in this report, the frequency of abruptio placentae more recently at Parkland Hospital was decreased by one half. Even so, abruptio placentae still accounted for 14% of all stillbirths.

In this report the answers to two questions especially have been sought: what accounted for the favorable reduction overall in the frequency of severe abruptio placentae so severe as to kill the fetus? and what might be done to reduce it further?

Material and methods

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The term abruptio placentae has been applied to lesions ranging from focal, marginal placental sepa-

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rations of minimal dimensions and causing little or no impairment of fetal well-being to detachments so extensive that placental function is inadequate for fetal survival. In the first circumstance aggressive intervention is unlikely to influence outcome unless the management itself adversely affects mother, fetus, or both. Moroever, it is not always possible, even in retrospect, to establish precisely either the extent or the duration of abruptio placentae simply by examining the delivered placenta. However, one well-defined category of major clinical importance is identifiable, namely, placental separation with loss of placental function so great that the fetus dies in utero. We have so defined severe abruptio placentae.

All Parkland Memorial Hospital inpatient, outpatient, emergency department, and family planning clinic records, and, when appropriate, records from other hospital and private physicians have been reviewed for the 207 cases of severe abruptio placentae cared for from 1974 through 1989. Most all of the laboratory studies were performed in the Obstetric Hematologic Research Laboratory using standard methods.

Results

The frequency of abruptio placentae so severe as to kill the fetus decreased from one in 420 deliveries during 1956 through 1969 to one in 830 during 1974 through 1989. The roles, if any, of the following factors in bringing about this reduction are considered.

High parity. In the 1956 through 1969 study, the risk of severe abruptio placentae was greatest among women of very high parity.1 Indeed, the frequency of severe abruptio placentae among women whose pre-

Table I. Characteristics of 207 cases of severe abruptio placentae, 1974 to 1989

		. Total deli	veries	
Ethnic group	No. of cases of abruptio placentae	No.	%	Frequency
All	207	171,787	100	1:830
Black	115	68,264	40	1:594
White	53	46,442	27	1:876
Latin American	36	53,026	31	1:1473
Other	. 3	4,055	2	1:1352

Black versus white, p = 0.02.

Latin American versus black and white, p = 0.00002.

delivery parity was ≥ 7 was 1 in 120, or seven times that for women whose parity was 1. In the previous study 37 of the 201 cases, or 18%, involved women with a parity of ≥ 7 compared with only two instances, or 1%, more recently, reflecting the dramatic decrease in the frequency of high parity that has been achieved for the general obstetric population at Parkland Hospital.

If the risk of severe abruptio placentae for each parity had remained the same as in the earlier period, the marked reduction in the frequency of high parity alone would have resulted in a decrease in the frequency from 1 to 420 to 1 in 576 deliveries, or 37%.

Some investigators have concluded that high parity is not a risk factor for severe abruptio placentae, but they were analyzing typically an obstetric population in which high parity was rare and, even though numeric differences were identified, these were not considered to be significant.²

Race. The frequency of severe abruptio placentae more recently was quite different for black, white, and Latin American women. While the mean frequency for all races was 1 in 830, it varied from 1 in 594 for black women and 1 in 876 in white women to only 1 in 1473 among Latin American women. These differences are highly significant (Table I). Age and parity among the three groups differed only slightly: Mean predelivery parities for black, white, and Latin American women were 1.12, 1.02, and 2.0, respectively, and mean ages were 21.5, 22.2, and 24.6 years respectively. Therefore the addition of a sizable number of Latin American women to the general obstetric population would serve to decrease the overall frequency of abruptio placentae so severe as to kill the fetus, and, indeed just that has occurred during more recent years at Parkland Hospital, as shown in Fig. 1.

In our 1956 through 1969 study, evaluation of the impact, if any, of race was not attempted because most of the women (83%) with severe abruptio placentae were black, but so were 76% of the general obstetric population, at that time. During the same period only 7% of women delivered at Parkland Hospital were Latin American, compared with 32% during 1974 through 1989. In retrospect, the 7% of Latin Americans

in the earlier study accounted for only 2.6% of the cases of severe abruptio placentae, in keeping with the finding that severe cases are much less common among Latin American than black women.

Racial differences in the predisposition to severe abruptio placentae have not been considered in most publications. The review by Naeye et al.3 of 53,518 pregnancies from 12 hospitals between 1959 and 1966 is an exception. Their analyses of cases of abruptio placentae and perinatal death provided by the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke led them to conclude that race had no significant influence on perinatal deaths caused by abruptio placentae. These investigators did not specify the races considered, but presumably they were predominantly white and black. In our recent experiences severe abruptio placentae was less common among whites than blacks, but the level of significance (p = 0.02) was not great, whereas for Latin Americans compared with black plus white women the difference was highly significant (p = 0.00002). Our study involved 171,780 deliveries, 54,326 of which were in Latin American women.

The intriguing, recently reported findings of Kleinman,⁴ that neonatal infant mortality among women of Mexican origin was less than one half that for black women, would suggest, at least, that pregnancy complications that compromise fetal well-being are likely to be less frequent or less intense among Latin American women when compared with black women.⁴

Prevalence of sickle cell trait. The frequency of hemoglobin S was determined among the 115 black women who more recently had severe abruptio placentae. There were no instances of sickle hemoglobinopathy, but sickle cell trait was identified in 5, or 4.2%. During these study periods, erythrocytes from 47,562 black women who attended prenatal and family planning clinics were screened for hemoglobin S; the result was positive in 8.1%.

Maternal sickle cell trait was not a factor in the genesis of severe abruptio placentae, nor did it intensify morbidity.

Maternal hypertension. Hypertension was defined as blood pressure repeated >140/90 mm Hg for >6 hours. If hypertension was identified remote from pregnancy it was classified as chronic. Blood pressure recordings remote from pregnancy were available in 97% of cases.

The frequency of hypertension from all causes was similar in both studies, averaging 52% in the more recent study and 47% earlier compared with 9.6% and 11%, respectively, in the general obstetric population. Acute hypertension was recently more common (33% vs 22% earlier); however, 44% of the general obstetric population delivered more recently were nulliparous and therefore at increased risk of pregnancy-induced hypertension, compared with 25% who were nulliparous in the earlier study. Among women with severe abruptio placentae more recently, the frequency of hypertension was 36% among white women, 49% in Latin Americans, and 59% in black women, compared with 9.8%, 10.8%, and 12%, respectively, in the general obstetric population who were delivered during the same period.

Whereas these observations confirm previous reports of a markedly increased frequency of hypertension in cases of abruptio placentae,1,2,5 they do not provide an explanation for the remarkably lower frequency of severe abruptio placentae among Latin American women compared with black women. The difference does not appear to be attributable to a much lower prevalence of hypertension and therefore a reduced predisposition to abruptio placentae in Latin Americans compared with the black general obstetric population because the frequency of hypertension in the two obstetric populations differed only slightly.

Recurring abruptio placentae. Eighty-two of the women in the current study are known to have conceived again 133 times; 20 of the subsequent pregnancies (15%) terminated in abortion, including two tubal pregnancies.

Fourteen (12%) of pregnancies in which birth weight was ≥500 gm were complicated again by abruptio placentae that was fatal to 8 (7%) of the fetuses. One mother (Case 6, Table II) died of postpartum complications that included severe hypertension, renal failure and respiratory distress, whereas in the earlier study all mothers survived. Thus fetal outcome was no better than in the period 1956 through 1969, during which 11% of the 139 subsequent pregnancies terminated with another abruptio placentae; in 7% the placental separation again proved fatal to the fetus, and maternal outcome was worse.

To try to identify, at least in retrospect, how these deaths from subsequent abruptio placentae might have been prevented, the 14 repeat instances were carefully reviewed. Summaries are provided in Table II. The

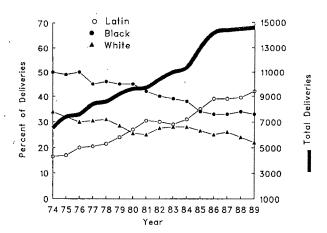


Fig. 1. Racial distribution of obstetric population at Parkland Memorial Hospital, 1974 through 1989.

opportunity to evaluate mother and fetus and intervene quickly while the fetus was still alive was important in the favorable outcome in 5 of the 6 cases of recurring abruptio placentae in which the infant survived.

Seventeen of the women who were again pregnant after a previous abruptio placentae that was fatal to the fetus were hospitalized prophylactically antepartum during the more recent study period for close monitoring of fetal and maternal well-being and, whenever premature placental separation was suspected, to accomplish delivery rapidly and hopefully, obtain a healthy infant. In 14 instances a live-born infant who survived was delivered without evidence of premature placental separation. In two of the pregnancies (cases I and 5, Table II) prompt identification of abruptio placentae causing fetal distress during hospitalization of the mother in the high-risk unit led to immediate cesarean delivery with infants surviving. However, in the third case (case 8) the fetus was stillborn even though the mother was hospitalized for 5 weeks, during which nonstress tests three times a week were reactive and frequent ultrasonographic evaluations demonstrated satisfactory fetal growth and an appropriate volume of amnionic fluid. This was also the unfortunate outcome in three other pregnancies in which abruptio placentae that was fatal to the fetus occurred while the mothers were hospitalized in the high-risk unit because of some complication other than previous abruptio placentae, usually mild to moderate pregnancy-induced hypertension. In two of these cases the nonstress test was reactive when performed <10 hours before fetal heart activity was discovered to be absent.

Out of 133 pregnancies subsequent to abruptio placentae fatal to the fetus, the loss of 15% of fetuses through abortion, 7% by stillbirth, and the death of one of the 83 mothers precludes any degree of optimism for subsequent reproductive attempts.

Table II. Fourteen recurrences in 12 women after abruptio placentae fatal to fetus

	z zz. rodricen recarrences n	1 12 women after abruptio pia		
Case No.	Index abruptio placentae fatal to fetus	Subsequent abruptio placentae	Follow-up	Comment .
1 2	Gravida 1, abruptio placentae, severe DIC, 605 gm stillborn infant Gravida 1, abruptio placentae, 1590 gm stillborn	Gravida 2, admitted to high-risk unit at 32 wk, borderline hy- pertension, small abruptio placentae next day, prompt CS 2210 gm healthy infant Gravida 2, 36 wk, severe PIH, fetal distress, abruptio placen-	—.	Recurrent abruptio placentae in spite of normal NST and ultrasonographic eval- uation on day of abruptio placentae Prompt delivery saved fetus
	infant	tae CS 1870 gm healthy infant		
3	Gravida 1, 28 wk, abruptio placentae 1180 gm still-born infant	Gravida 2, 33 wk severe hypertension, fetal distress, CS 1850 gm stillborn infant	Gravida 3, spontaneous abortion Gravida 4, severe PIH, thrombocytopenia, hepatic dysfunction, stillbirth without abruptio placentae Gravida 5, 3380 gm healthy infant by repeat CS at 37 wk, no hypertension	Should last pregnancy have been delivered earlier "prophylactically"?
•	Gravida 2, PIH, abruptio placentae, DIC, 2365 gm stillborn infant Gravida 3, abruptio placen	Gravida 3, abruptio placentae at 36 wk, PIH, fetal distress, CS 2410 gm healthy infant Gravida 4, admitted to high-risk	. -	Evaluation in clinic was nor- mal on day before recur- rent abruptio placentae Recurrent abruptio placentae
	tae, severe DIC, 3000 gm stillborn infant	unit at 37 wk, abruptio pla- centae 2 days later CS, 3560 gm healthy infant		at 37 wk (should elective delivery have been planned earlier?)
6	Gravida 1, 36 wk, PIH and abruptio placentae, CS, 2270 gm stillborn infant	Gravida 2, mild chronic hypertension, abruptio placentae 29 wk, 1150 gm stillborn infant by repeat CS	Severe hypertension, uremia, hemolysis, intense thrombocyto- penia, ARDS. Mother had fatal hemorrhage 15 days post partum when Swan-Ganz catheter	Some clinical features of hemolytic-uremic syn- drome
			eroded pulmonary vessel	
7	infant, massive hemor- rhage	Gravida 4, 36 wk, 2420 gm fetus, 2 hr after onset of pain severe DIC	- ,	Pregnancy considered nor- mal at clinic visit 2 days earlier
8	Gravida 4, 30 wk, abruptio placentae, 1415 gm still- born infant	Gravida 6, admitted to high-risk unit at 30 wk; during next 5 wk appropriate fetal growth and reactive NSTs 3/wk, nor- motensive, DIC, 2115 gm stillborn infant	Third pregnancy- growth-retarded in- fant did well; fifth pregnancy, 1965 gm stillborn infant at 35 wk, cord accident	Prolonged hospitalization with close observation and frequent reactive NST did not prevent stillbirth and severe DIC from recurrent abruptio placentae in 6th pregnancy
9	Gravida 1, 36 wk, fetal growth retardation, abrup- tio placentae, 1270 gm still- born infant	Gravida 4, 41 wk, abruptio placentae, 2560 gm stillborn infant Gravida 5, spontaneous labor at 33 wk, healthy 2245 gm infant, 20% abruptio placentae		Did not return to prenatal clinic during last 8 wk of fourth pregnancy
10	Gravida 4, abruptio placentae, DIC, 2235 gm infant stillborn	Gravida 5, abruptio placentae at 33 wk, DIC, mild chronic hy- pertension, 2080 gm stillborn infant	Mild chronic hyper- tension	Onset of abdominal pain 7 hr before seeking care; would more prompt care have prevented stillbirth?
11	Gravida 3, PIH, abruptio pla- centae, DIC, stillborn in- fant at 26 wk	Gravida 4, abruptio placentae 31 wk, fetal distress, CS, 1480 gm healthy infant	· _	Prompt delivery saved fetus
12	Gravida 3, 32 wk, PIH, abruptio placentae, CS, stillborn infant	Gravida 4, 29 wk, abruptio placentae, 725 gm growth-retarded stillborn infant Gravida 5, 24 wk, third fatal abruptio placentae, 620 gm stillborn infant	Tubal sterilization after fifth pregnancy; nor- motensive 1 yr after last pregnancy	Before first abruptio placen- tae fatal to fetus, she expe- rienced severe pre- eclampsia, fetal distress, and partial abruptio pla- centae at 32 wk; 1270 gm fetus was delivered promptly by CS and sur- vived

The experiences with hospitalized women who previously had severe abruptio placentae, to try to monitor fetal well-being closely and to be able to effect delivery rapidly if abruptio placentae should recur, admittedly are limited, but until there is more evidence of benefit, the expense alone is hard to justify. Instead, it is urged that effective education be provided to the pregnant woman who has had severe abruptio placentae previously, to her family, and to all of the members of the obstetric staff who participate in her care. The serious risks associated with a subseqent pregnancy, the need to see care immediately, day or night, whenever abdominal pain, low back pain, or vaginal bleeding develops, and the need at times for immediate delivery require emphasis.

Is a recommendation of deliberate preterm delivery justified on the basis that during two periods of time a previous abruptio placentae fatal to the fetus predestined recurrent placental separation and stillbirth for 7% of subsequent pregnancies? If so, how remote from term? Delivery of all such pregnancies once the fetus achieved a weight of 2000 gm would have yielded a live-born infant in one half the cases in both our studies. At the same time prematurity would have been imposed deliberately on nearly 90% of pregnancies in which abruptio placentae fatal to the fetus was not destined to recur. Even so, preterm delivery should be considered once serious complications from prematurity are unlikely. By so doing, perhaps two of the eight fetal deaths from recurring abruptio placentae in the current study and three of the 10 deaths in the earlier study might have been avoided. Moreover, maternal morbidity and mortality even with cesarean delivery are likely to be less when delivery is accomplished under well-controlled conditions rather than the hectic state often imposed by abruptio placentae with fetal and maternal distress.

Trauma. In only three instances was abdominal trauma implicated in the genesis of abruptio placentae fatal to the fetus. In each case, even though the mother was wearing a seat belt, the abdomen struck the steering wheel forcefully during an auto accident, causing in two of the cases a sizable laceration of the placenta with severance of overlying fetal vessels and lethal fetoplacental-to-maternal hemorrhage. Both cases are considered further under "Fetoplacental hemorrhage."

Even though Parkland Hospital is a major trauma center for the greater Dallas region, in both studies external trauma was rarely identified as the probable cause of abruptio placentae fatal to the fetus. External trauma was implicated etiologically only five times in our two studies, involving a total of 408 cases so severe as to kill the fetus. During this same time, births at Parkland Hospital exceeded 250,000. These findings deserve emphasis for medicolegal reasons alone.

Fetoplacental hemorrhage. Three instances of massive fetoplacental-to-maternal hemorrhage were identified. Two, mentioned above, were in women who struck the steering wheel with enough force to lacerate the placenta, and the third had a septate uterus. Red blood cells resistant to acid elution, equivalent to 30% to 70% of the normal red blood cell content of the fetoplacental circulation, were identified in the maternal circulation.

In 78 additional cases of severe abruptio placentae, erythrocytes containing hemoglobin F were sought in smears of maternal blood. In 61 instances (80%) no hemoglobin F-containing cells were seen at inspection of \geq 4000 red blood cells per slide. In the 17 instances in which smears were positive, the volumes of red blood cells rich in hemoglobin F in the maternal circulation were estimated according to the formula of Mollison⁶ to be <10 ml. Most likely, red blood cell loss of this magnitude from the fetoplacental circulation into the maternal circulation did not contribute to fetal death.

These observations imply that hemorrhage from the fetoplacental circulation is most likely to be a major factor in fetal death in circumstances in which the abdomen has been subjected to considerable force and, in turn, the placenta has been actually lacerated, as in the two trauma cases cited above. Otherwise, life-threatening fetoplacental-to-maternal hemorrhage did not appear to be a factor in fetal death.

Premature rupture of membranes. In two instances of gross hydramnios, abruptio placentae with brisk vaginal bleeding soon followed spontaneous rupture of the membranes and the rapid escape of massive volumes of amniotic fluid. An anencephalic fetus in one case and a hydrocephalic fetus in the other died in utero. Most likely the rapid decrease in the size of the uterine cavity, including the retroplacental uterine surface to which the placenta had been attached, served to shear off the placenta, just as delivery of the fetus normally does. In our earlier study we identified one case of marked hydramnios with rupture of membranes and acute abruptio placentae. Preterm premature rupture of the membranes in the absence of gross hydramnios was identified only twice in these 207 cases.

In at least three reported studies preterm premature rupture of the membranes has been implicated in the genesis of abruptio placentae, even in the absence of gross hydramnios or other recognized predisposing factors. 7-9 In the absence of hydramnios, however, we found little evidence that premature rupture of the membranes is likely to result in severe abruptio placentae. To reduce maternal discomfort and enhance the quality of labor in pregnancies complicated by severe hydramnios, it has been our policy as prophylaxis to drain off slowly approximately one half the estimated volume of fluid transabdominally. This policy has minimized the risk of sudden, massive decompression of the uterus and severe abruptio placentae after spontaneous rupture or transvaginal amniotomy.

Maternal folate and iron deficiencies. A flurry of

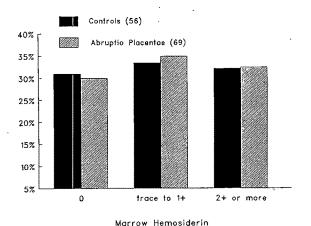


Fig. 2. Maternal storage iron identified histochemically as hemosiderin.

reports, in the 1960s and 1970s especially, implicated folic acid deficiency in the genesis of abruptio placentae, but several other studies failed to affirm a relationship. Nonetheless, the impression that abruptio placentae can be a consequence of folic acid deficiency has persisted in some quarters.

We investigated still another characteristic feature of abnormal folate metabolism, namely, maternal red blood cell macrocytosis in women with severe abruptio placentae. Mean corpuscular volume was measured electronically on a sample of blood obtained before transfusion from 175 women with abruptio placentae sufficient to kill the fetus; the results were compared with those obtained from 186 control women admitted for routine delivery. Neither group was given supplemental folic acid prenatally. The mean corpuscular volume averaged 89.0 fl for the women with severe abruptio placentae compared with 89.1 fl for the control group.

Since coincidental iron deficiency might mask macrocytosis from folate deficiency, iron stores of 64 women with severe abruptio placentae and 56 who were admited for routine delivery were evaluated histochemically as hemosiderin in bone marrow aspirates obtained during earlier extensive studies of folate and iron metabolism in late pregnancy. As shown in Fig. 2, iron deficiency was no more common in women with severe abruptio placentae than in the general obstetric population.

Neither folic acid nor iron deficiency is an important factor in the genesis of severe abruptio placentae.

Uterine anomaly or tumor. In two instances of severe abruptio placentae a septate uterus was observed. The only women in whom uterine leiomyomas was identified underwent hysterectomy 1 month after experiencing abruptio placentae that was fatal to the fetus. None of the myomas were submucous.

As in our earlier study, evidence is lacking to impli-

cate uterine anomalies or myomas as a frequent cause of severe abruptio placentae. Rice et al., 10 however, recently reported a frequency of 10.8% for abruptio placentae of all severities in pregnancies of women with leiomyomas, but even more recently Davis et al. 11 reported no instances of abruptio placentae in an anterospective study of 85 pregnant women with uterine leiomyomas. The results of our studies are in agreement with those of Davis et al.

Comment

The reduction by almost 50% in the frequency of severe abruptio placentae at Parkland Hospital from 1 in 420 deliveries during 1956 through 1969 to 1 in 830 from 1974 to 1990 can be attributed for the most part to two major changes observed in the obstetric population. There has been a dramatic decrease in the number of women at increased risk of severe abruptio placentae because of very high parity (≥7) and a marked increased in the number of pregnancies among Latin American women during the more recent study period. For reasons not yet identified Latin American women had severe abruptio placentae much less often (1:1473) than did white women (1:876) and especially black women (1:594). Hemoglobin S was very uncommon in black women with severe abruptio placentae and, when present, it did not appear to enhance maternal morbidity.

In both the earlier and the more recent study periods, hypertension was identified in nearly one half the women with severe abruptio placentae.

Abruptio placentae recurred in 12% of subsequent pregnancies, almost identical to the recurrence rate in the 1956 through 1969 study. It is discouraging that in more than half of the recurrences abruptio placentae again proved fatal to the fetus. The need for education of all parties involved—patient, family, and all who participate in obstetric care—is emphasized. Such education should help achieve a favorable outcome, as well as reduce the risk of medicolegal entanglements.

External trauma was recognized rarely in the genesis of abruptio placentae fatal to the fetus. Three instances involved auto accidents with a forceful blow to the abdomen. In two of the three cases the placenta was grossly lacerated with severe hemorrhage from the fetoplacental circulation, sufficient of itself to cause fetal death. Otherwise, evidence of fetoplacental-to-maternal hemorrhage that was life-threatening to the fetus was lacking.

Premature rupture of the membranes was implicated in the genesis of severe abruptio placentae in two cases of severe hydramnios with grossly ruptured membranes and rapid loss of huge volumes of amniotic fluid. Preterm prematurely ruptured membranes in the absence of hydramnios was identified rarely. Uterine leiomyomas and uterine anomalies from faulty müllerian duct fusion were rare in the 207 cases, as they were in the earlier 201 cases of severe abruptio placentae.

Abruptio placentae so severe as to kill the fetus persists as a prominent cause of fetal death.

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A rapid visual test for predicting fetal lung maturity

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A rapid bedside test has been devised that enables an untrained observer to predict (p < 0.001) when amniotic fluid will be ≥ 0.15 at an optical density of 650 nm, lecithin/sphingomyelin ratio will be ≥ 2.0 , or when phosphatidylglycerol will be present. By a visual comparison of the turbidity of unspun amniotic fluid against positive (mature) or negative (immature) controls, technicians and resident physicians who had had no special training were able to classify correctly 87.2% (82/94) of unknown amniotic fluid samples. The sensitivity of the new test is 90.8% (58/65); the specificity is 70.3% (23/29). Thus, when more sophisticated methods are not readily available, we believe that this easily performed and accurate test can provide supplemental or preliminary data for patient management. In remote geographic areas our method could serve as the primary source of information about fetal lung maturity. (Am J Obstet Gynecol 1991;165:1351-3.)

Key words: Fetal lung maturity, amniotic fluid turbidity, visual test

After having done >2500 determinations of optical density at 650 nm (OD₆₅₀) on spun amniotic fluid during

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the past 10 years, we have found that fetal pulmonary maturity test results often could be visually predicted a priori. Amniotic fluid samples that had an $OD_{650} \ge 0.15$, a lecithin/sphingomyelin (L/S) ratio ≥ 2.0 , or phosphatidylglycerol present were usually more turbid than were immature specimens.

Because commonly used tests for lung maturity, such as those for L/S ratio and phosphatidylglycerol,¹ require laboratory support that may not be readily available in all health care settings, simpler methods have been sought by many investigators.² We felt obligated

Table I. Amniotic fluid samples with unspun $OD_{650} > 0.50$ and < 0.80

No. of subjects	Unspun OD ₆₅₀	Fetal lung maturity indices
26	>0.50 and <0.80	Mature
22	>0.50 and <0.80	Immature

Table II. 2×2 Contingency with spun OD₆₅₀, L/S ratio, and phosphatidylglycerol analysis as clinical standards for visual observations

Clinical standard of OD ₆₅₀ , L/S ratio, and phosphatidylglycerol	Mature	Immature
Mature	59	. 6
Immature	6	23

 $[\]chi^2$ (1 df) test statistic, 46.5 (p < 0.001).

to test our hypothesis that an untrained observer could predict the outcome of fetal pulmonary maturity indices by comparing an unspun sample of amniotic fluid with known standards, one mature and the other immature.

Material and methods

In the first phase of the study 167 amniotic fluid samples were obtained by transabdominal amniocentesis for various indications and at various gestational ages. Within 2 hours of collection, OD₆₅₀ measurements were done on a Beckman DB spectrophotometer on unspun fluids with 1 cm light-path cuvettes standardized against distilled water. After the unspun optical density was recorded, samples were spun at 3200 revolutions/min (2000 g) in an International model PR-J refrigerated centrifuge for 10 minutes. The spun OD₆₅₀ was then recorded, and L/S ratios and phosphatidylglycerol analyses were performed as previously described. Specimens containing gross meconium, bilirubin, or hemolyzed blood were excluded.

From these data, positive (mature) and negative (immature) controls were determined. Because the OD₆₅₀ on unspun amniotic fluid was always \geq 0.80 when spun OD₆₅₀ was \geq 0.15, L/S ratio was \geq 2.0, or phosphatidylglycerol was present, the control for maturity was designed to mimic amniotic fluid with that level of turbidity. Because an unspun amniotic fluid OD₆₅₀ \leq 0.50 invariably was found when spun OD₆₅₀ < 0.15, L/S ratio was <2.0, or phosphatidylglycerol was absent, the immature control was designed to mimic the clearer fluid. The standards were made by dissolving purified agar in distilled water, then dispensing 8 to 10 ml of the solutions into capped Vacutainer tubes. The positive control contained 3% agar, whereas the negative

control contained 0.75% agar. The tubes were then autoclaved for 15 minutes at 15 pounds per square inch of steam pressure. After the caps were cooled, they were firmly placed and sealed with tape.

In the validation phase of the study 94 samples of amniotic fluid were collected from new patients and then compared visually with the two controls. The predictions were recorded as being mature, immature, or indeterminate. After centrifugation, spun OD_{650} , L/S ratios, and phosphatidylglycerol analyses were performed to serve as clinical standards.

Results

During the first phase of the experiment, 167 samples were analyzed. In 87 of these specimens, the unspun OD_{650} was ≥ 0.80 and the maturity indices were positive. In 32 samples the unspun OD_{650} was ≤ 0.50 and the maturity indices were negative. Thus in 119 (87 + 32) samples the standards were either mature or immature. In 48 (167 – 119) amniotic fluid specimens the outcomes were mixed; i.e., if the unspun OD_{650} was >0.50 but <0.80, the spun OD_{650} , L/S ratio, or phosphatidylglycerol could be either mature or immature (Table I).

In the validation phase, amniotic fluid samples from new patients were judged by untrained observers (technicians and resident physicians) to be positive or negative when compared with the two controls. After the evaluations, the usual lung maturity testing was performed to provide confirmation. Sixty-five amniotic fluid samples judged visually were thought to be positive. Subsequent testing confirmed that 59 of these samples were mature (Table II). Twenty-nine fluid specimens were judged visually to be negative, and 23 of those opinions were confirmed by testing. When the results were evaluated as a 2 × 2 contingency in which the laboratory indices were considered the clinical standards against which the observations were compared, the results of the χ^2 test with 1 df were highly statistically significant (p < 0.001). Thus we conclude that untrained observers who use this rapid test can accurately discriminate mature from immature amniotic fluid. The sensitivity of the test was 90.8% (59/65), the specificity was 79.3% (23/29), the positive predictive value was 90.8% (59/65) (for the validation study in which the prevalence of mature amniotic fluid was 0.69 [65/94]), and the predictive value negative was 79.3% (23/29).

Comment

The L/S ratio and phosphatidylglycerol analysis are among the most widely used tests for determining fetal pulmonary maturity. However, they require complex methods that limit their availability. Thus a number-of simpler methods have been reported. Our own re-

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search laboratory introduced one of those alternatives, the spun OD₆₅₀, which has been shown to compare favorably with the L/S ratio and phosphatidylglycerol analysis in predicting the likelihood of respiratory distress syndrome in newborns.³⁻⁶ However, even spun optical density determinations require centrifugation. To improve clinical access for evaluating amniotic fluid to determine fetal pulmonary maturity, we have developed a rapid, easily performed test that needs no laboratory support. Our method requires only two turbidity tubes and a needle for amniocentesis. We have not yet validated amniotic fluid specimens obtained from vaginal pools.

Although we do not suggest that our rapid test should displace accepted laboratory standards for determining fetal lung maturity, we do believe that our simple method can be a useful preliminary assessment in many hospitals. Furthermore, in remote locales that do not enjoy the advantages of more sophisticated methods, our test (which has a 91% sensitivity and thus a 9% false-positive rate that must be considered in any clin-

ical decision-making) can provide information about pulmonary maturity when no other test is available.

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Fetal abdominoscrotal hydrocele

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A case of abdominoscrotal hydrocele detected initially as an intraabdominal cyst on antenatal ultrasonography is described. Abdominoscrotal hydrocele should be included in the differential diagnosis of antenatally recognized intraabdominal cysts and abdominal masses in the newborn. (AM J OBSTET GYNECOL 1991;165:1353-5.)

Key words: Abdominoscrotal hydrocele, fetus, ultrasonography

Antenatal maternal ultrasonography is an ideal way of evaluating pregnancy from both the obstetric and fetal points of view. Even though the precise nature of the abnormality may not be clear antenatally, antenatal recognition leads to appropriate postnatal assessment and treatment. Abdominoscrotal hydrocele is a rare condition, and there are no reports of antenatal detec-

tion of this disorder.^{1, 2} We report a case of abdominoscrotal hydrocele recognized as an intraabdominal cyst before 30 weeks of gestation that was managed surgically at 5 days of age.

Case report

Routine abdominal ultrasonography in a 28-year-old, gravida 11, para 10 woman at 22 weeks' gestation revealed a prominent cystic mass in the fetal abdomen that was thought to be a distended urinary bladder. Subsequent ultrasonography at 30 and 34 weeks' gestation revealed a fetal abdominal cyst of 5.7×4.5 cm (Fig. 1). The cyst was distinct from the urinary bladder, and the kidneys were normal. There was no evidence of any alimentary tract abnormality, and the exact na-

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Fig. 1. Ultrasonography at 30 weeks' gestation.

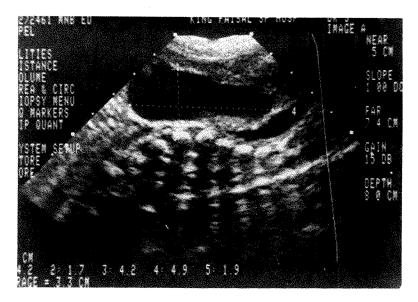


Fig. 2. Postnatal ultrasonography showing cystic mass of abdominoscrotal hydrocele.

ture of the fetal cyst was not clear, although mesenteric cyst was considered a possibility.

The baby was born at 37 weeks' gestation by cesarean section; the birth weight was 3150 gm. Abdominal examination revealed an undistended abdomen with a soft suprapubic mass with ill-defined margins. There was also a large left inguinal swelling that could be reduced into the peritoneal cavity and was thought to be a hernia. The testes were normal. Postnatal ultrasonography and abdominal x-ray film confirmed the presence of the suprapubic cystic mass (Fig. 2). A precise diagnosis was not made, but as a result of the "hernia" early surgery was undertaken at 5 days of age. On exploration of the left inguinal canal a large cystic swelling was noted that extended into the abdominal cavity. Laparotomy was performed through a separate iliac

fossa incision when it was obvious that the "cyst" was an abdominoscrotal hydrocele that was retroperitoneal in position. The hydrocele sac extended posteriorly just lateral to the bladder, to which it was adherent. It was separated from the testicular vessels and vas and was then removed; the procedure was completed with an orchiopexy. The postoperative course was uneventful, and the baby was discharged on the fourth postoperative day. He was well and thriving at 6 months of age and had no sign of residual abdominoscrotal swelling. The testes were normal.

Comment

Abdominoscrotal hydrocele is essentially a hydrocele of the tunica vaginalis that has an inguinoscrotal and an abdominal component. Even though various etiologic theories about this condition have been proposed, the likely mechanism is that increased pressure from overdistention of an inguinoscrotal lesion produces the proximal (abdominal) herniation.^{1,2} It is also possible that the hydrocele may originate within the abdominal cavity and may extend or prolapse into the inguinoscrotal region.

Our case was unique in that the abdominal component was recognized antenatally, possibly at 22 weeks' gestation but certainly by 30 weeks. Although testicular descent normally takes place at 28 to 30 weeks' gestation, it is logical to assume that the hydrocele developed primarily within the abdominal cavity. Because the testes were in the normal scrotal position at birth, it is also likely that an abdominal hydrocele became an abdominoscrotal hydrocele by virtue of increased pressure within the abdominal component and downward displacement. It would also appear that with the increasing use of ultrasonography, both antenatally and postnatally, an "abdominal" hydrocele without an inguinoscrotal component may be seen.

Although abdominoscrotal hydrocele is extremely rare, it must be considered in the differential diagnosis of a lower abdominal cystic mass in a fetus. Hydronephrosis and hydroureter also may be seen with an abdominoscrotal hydrocele.

During postnatal assessment, ultrasonography is the most appropriate investigation. In a newborn with an inguinoscrotal swelling, the abdomen should be carefully palpated. If a lower abdominal cystic swelling is palpable, pressure on the mass may produce a scrotal swelling or make it more obvious and thus lead to a clinical diagnosis of abdominoscrotal hydrocele. Treatment of this condition is total excision, and récurrence is rare unless it is not totally excised.

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Editors' note

The American Journal of Obstetrics and Gynecology introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Increased serum levels of macrophage colony-stimulating factor in ovarian cancer

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Macrophage colony-stimulating factor is a cytokine that stimulates proliferation and differentiation of phagocytic cells. Macrophage colony-stimulating factor is produced by ovarian epithelial cancer cell lines and might provide a useful serum marker for the disease. Among sera from 69 patients with clinically apparent epithelial ovarian cancer, 47 (68%) had at least 2.5 ng/ml macrophage colony-stimulating factor, whereas only two of 80 apparently healthy donors (2.5%) had a comparable elevation of macrophage colony-stimulating factor. Circulating levels of macrophage colony-stimulating factor did not correlate with serum levels of CA 125. Moreover, 14 of 25 ovarian cancer patients (56%) with clinically evident disease and normal levels of CA 125 (<35 U/ml) had elevated levels of macrophage colony-stimulating factor. Among 29 patients with serum CA 125 levels <35 U/ml before positive surgical surveillance procedures, 9 (31%) had at least 2.5 ng/ml macrophage colony-stimulating factor. Elevated levels of macrophage colony-stimulating factor were also found in patients with carcinomas from other primary sites and in 31% of 134 patients with benign diseases. If intercurrent benign disease can be taken into account, macrophage colony-stimulating factor deserves further evaluation in combination with CA 125 in monitoring ovarian cancer. (AM J OBSTET GYNECOL 1991;165:1356-62.)

Key words: Tumor marker, macrophage colony-stimulating factor, cytokines, ovarian carcinoma, renal failure

Tumor cells produce growth factors, some of which may regulate the proliferation of cancer cells by autocrine and paracrine pathways.1-7 The aberrant overexpression of a trophic factor by cancer cells can relate to gene amplification or to a genetic translocation that brings a growth factor gene under the control of different regulatory elements. Interestingly, colony-stimulating activity has been detected in a number of nonhematologic tumors.6,7 Concomitant with the expression of colony-stimulating factor (CSF) activity, higher levels of circulating leukocytes have been seen in some of these patients.4.5 Both clinical observations and studies of human tumor xenografts in nude mice indicate that cytokines can be produced constitutively by malignant cells.7 Although the importance of these factors remains uncertain, cytokine levels in serum may provide markers that can be used to detect tumor growth and to monitor treatment response.

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In a recent study we reported that each of six ovarian cancer cell lines secreted macrophage CSF into the culture medium in the absence of any external stimuli.8 Although lymphoid and myeloid cell lines failed to produce detectable amounts of macrophage CSF, a limited number of breast cancer cell lines secreted the factor in significant quantities. The presence of macrophage CSF was confirmed by immunologic and biologic assays and by analyzing total cellular ribonucleic acid for macrophage-CSF-related transcripts.8 Kacinski et al.9 have detected the expression of macrophage CSF transcripts in ovarian carcinoma cells and ovarian adenocarcinomas express fms complementary transcripts and fms antigen.9, 10 Macrophage CSF also has been found in plasma from patients with ovarian, endometrial, mammary, and pulmonary carcinoma.11-13

Macrophage CSF is a dimeric glycoprotein of about 70 kd, it belongs to a group of cytokines that regulate the ontogeny of blood cells. Macrophage CSF selectively stimulates proliferation and differentiation of both monocyte precursors and mature macrophages. As a result of our earlier finding that macrophage CSF is produced by ovarian cancer cell lines ex vivo, we investigated whether patients with ovarian cancer have an increased level of macrophage CSF in serum. An earlier study reported that macrophage CSF levels were elevated in ovarian cancer patients that included a small subset of patients monitored longitudinally in and whom macrophage CSF was elevated

at recurrence without a simultaneous elevation of CA 125. We were especially interested in determining the frequency of macrophage CSF elevation in a larger number of ovarian cancer patients with normal levels of CA 125 to determine whether macrophage CSF and CA 125 are complementary tumor markers for ovarian cancer. Serum samples collected from patients with well-documented disease were analyzed with a specific radioimmunoassay for macrophage CSF. We have found that macrophage CSF levels are elevated in approximately two thirds of women with clinically apparent ovarian cancer and that macrophage CSF can complement CA 125 as a marker for the presence of residual disease. This confirms and extends the studies of Kacinski et al.9,13 This is the first study to determine macrophage CSF levels in a large number of patients with various benign diseases. The highest frequency of macrophage CSF elevation among patients with benign disease was found in those with renal disease. Elevations of macrophage CSF also were observed in sera from patients with nonovarian carcinomas, such as those of the lung and endometrium (as previously reported), 12.13 as well as in patients with cervical, colon, and breast carcinomas.

Material and methods

Clinical material. A total of 104 serum samples were obtained from 91 women with advanced epithelial ovarian cancer who were treated at the Duke University Medical Center. Protocols and procedures approved by the institutional review board were followed. Among the 91 ovarian cancers there were 82 serous, 3 mucinous, I endometrioid, I mixed, 2 undifferentiated, and 2 clear cell carcinomas. The tumor was well differentiated in 7 patients, moderately differentiated in 30, poorly differentiated in 25, and undifferentiated in 3. In 26 cases differentiation was not assessed. At diagnosis, 5 patients were in stage I, 2 were in stage II, 63 were in stage III, and 20 were in stage IV. In 1 patient with advanced disease the original stage was not known. Specimens were generally obtained at later intervals, when persistent or recurrent disease was clinically evident or before second-look surgical surveillance procedures. The 14 patients from whom sera were obtained preoperatively were all in stage III or IV and had average macrophage CSF levels of 3.23 ± 1.56 ng/ml. To obtain 69 serum specimens from patients with clinically evident disease, blood samples were drawn before initial exploration in 14 patients, within 7 days after initial laparotomy in 1 patient, and at later intervals when persistent disease could be demonstrated by physical examination or radiographic procedures in 54 patients. Serum samples from 35 patients with minimal amounts of residual disease were drawn before surgical surveillance procedures. When both panels were assembled, several thousand sera were re-

viewed to select specimens with <35 U/ml CA 125, in spite of the presence of clinically evident disease or the detection of tumor during a surgical surveillance procedure.

Blood samples were also collected from 134 women with benign diseases and from 81 women with adenocarcinomas of nonovarian origin who were treated at the Duke University Medical Center. In addition, blood was drawn from 80 apparently healthy women after informed consent was obtained. Serum was separated promptly, and aliquots were stored at -70° C until

Determination of serum macrophage CSF levels by radioimmunoassay. A polyclonal antiserum was prepared in rabbits by hyperimmunization against human recombinant macrophage CSF (Genetics Institute, Cambridge, Mass.). The antiserum used in these studies has been well characterized and does not cross react with other growth factors or with cytokines such as granulocyte-macrophage CSF, interleukin-2, or interferon gamma. Details of the assay conditions have been published elsewhere.8 The assay system can detect macrophage CSF concentrations as low as 10 pg per assay tube or 200 pg/ml. To minimize interassay variation, aliquots of individual sera samples from five normal controls were used as internal standards.

Determination of serum CA 125 levels by radioimmunoassay. All serum samples from ovarian cancer patients also were assayed for the presence of the tumor marker CA 125 with an immunoradiometric method. 16, 17 CA 125 levels were expressed in units per milliliter, and the concentrations of tumor-associated antigen were correlated with macrophage CSF by means of linear regression analysis.

Results

Macrophage CSF levels in healthy individuals and in patients with benign diseases. The mean concentration of macrophage CSF in sera from a population of apparently healthy women was 1.15 ± 0.65 ng/ml. Consequently, the upper limit for the normal range of serum macrophage CSF was chosen as 2.5 ng/ml to exceed 2 SDs from the mean. The data in Fig. 1 show the range of macrophage CSF levels in the control population. Only 2 of 80 apparently healthy donors (2.5%)had macrophage CSF levels (2.65 and 3.15 ng/ml) that exceeded 2.5 ng/ml. When sera were evaluated from patients with benign diseases, macrophage CSF was >2.5 ng/ml in 42 of 134 patients (31%) (Table I). Elevations of macrophage CSF were associated with several forms of benign disease, including renal disease (78%), acute infection (50%), pulmonary disease (44%), autoimmune disease (27%), and liver disease (23%). Benign diseases were categorized as autoimmune disorders, infectious processes, lung diseases, renal diseases, liver disease, and benign breast conditions. Au-

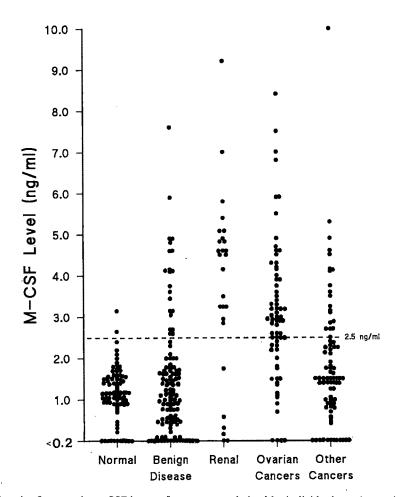


Fig. 1. Levels of macrophage CSF in sera from apparently healthy individuals, patients with benign diseases, ovarian cancer patients, and patients with a variety of nonovarian cancers. Each value is mean for triplicate determinations. Values in parentheses indicate number of samples analyzed.

Table I. Elevation of macrophage CSF (>2.5 ng/ml) in women with benign diseases*

Disease group	No. elevated per No. studied	%	Mean ± SD	Range (ng/ml)
Renal disease	21/27	78	3.58 ± 2.09	0.01-9.20
Hepatic disease	3/13	23	1.89 ± 1.99	0.01-7.60
Autoimmune disease	6/22	27	1.59 ± 1.35	0.01-4.90
Pulmonary disease	7/16	44	2.11 ± 1.68	0.01-4.90
Acute infection	5/10	50	2.88 ± 1.63	0.58-5.90
Benign breast disease	0/46	0	0.91 ± 0.66	0.01-2.00

*Elevations of macrophage CSF were associated with renal failure (n = 12), lupus nephritis (n = 4), polycystic kidney disease (n = 1), diabetic nephropathy (n = 1), nephrotic syndrome (n = 1), renal calculus (n = 1), cirrhosis (n = 2), hepatitis (n = 1), severe rheumatoid arthritis (n = 4), systemic lupus erythematosus (n = 2), chronic obstructive pulmonary disease (n = 3), Wegener's granulomastosis (n = 1), probable tuberculosis (n = 1), pulmonary alveolar proteinosis (n = 1), pulmonary mucormycosis (n = 1), pneumonia (n = 1), peritonitis (n = 1), staphylococcal sepsis (n = 1), urosepsis (n = 1), and fever of unknown origin (n = 1).

toimmune disorders included rheumatoid arthritis (n = 10); systemic lupus erythematosus (n = 4); Sjogren's syndrome (n = 2); multiple sclerosis (n = 3); myasthenia gravis (n = 1), psoriatic arthritis (n = 1), and autoimmune hemolytic anemia (n = 1). Infectious

processes included cellulitis (n = 2), pneumonia (n = 2), sepsis (n = 3), peritonitis (n = 1), and skin abscess and gangrene of the foot (n = 1). Benign lung diseases included chronic obstructive pulmonary disease (n = 6), asthma (n = 3), Wegener's granulomatosis (n = 1),

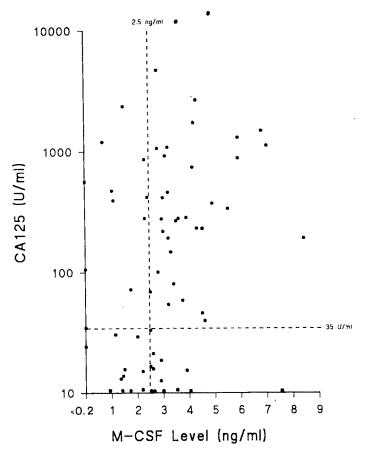


Fig. 2. Correlation between macrophage CSF levels and CA 125 levels in serum. Macrophage CSF and CA 125 were measured in serum samples obtained from ovarian cancer patients. Horizontal line demarcates 35 U/ml (value of CA 125 used to distinguish 99% of apparently healthy individuals). Vertical line indicates 2.5 ng/ml that exceeds mean level of macrophage CSF by 2 SD for healthy control population.

tuberculosis (n = 1), pneumonia (n = 1), histoplasmosis (n = 1), pulmonary alveolar proteinosis (n = 1), pulmonary mucormycosis (n = 1), and interstitial lung disease (n = 1). Renal diseases included lupus nephritis (n = 5), nephrotic syndrome (n = 3), nephrolithiasis (n = 3), polycystic kidney disease (n = 1), diabetic nephropathy (n = 1), renal stricture and obstruction (n = 1), and renal artery stenosis (n = 12). Liver disease included cirrhosis and hepatitis. Benign breast conditions included fibrocystic changes (n = 10), fibroadenomas (n = 7), chronic mastitis or abscess (n = 2), and various forms of dysplasia, hyperplasia, fibrosis, and duct ectasia (n = 10). Among the patients with renal disease, all 12 with renal failure had elevated levels of macrophage CSF. However, no elevations of macrophage CSF levels were observed among 46 patients with benign breast disease.

Serum macrophage CSF levels in ovarian cancer patients. As a result of our previous observation that ovarian cancer cell lines secrete macrophage CSF constitutively, it was of interest to find whether the levels

of this factor could be elevated in sera from ovarian cancer patients. Sera from 69 women with clinically apparent ovarian cancer were analyzed (Fig. 1). The mean value of macrophage CSF among these cancer patients was 3.02 ± 1.72 ng/ml, which was approximately 2.6-fold higher than the mean value of the control group. The values were distributed over a wide range from <0.2 to 8.4 ng/ml. Further analysis of the data indicated that 47 of the 69 cancer patients (68%) had macrophage CSF levels >2.5 ng/ml, which is an increase that is statistically significant (p < 0.001) when compared with levels in sera from healthy controls.

Comparison of macrophage CSF and CA 125. All serum samples from ovarian cancer patient were analyzed for CA 125 levels.17 In the current series 10 of the 69 samples had very low levels of CA 125 (<10 U/ml) and 12 samples had >1000 U/ml. To determine whether any correlation existed between CA 125 levels and macrophage CSF levels, paired values were plotted (Fig. 2). No definite relationship was found between serum levels of macrophage CSF and CA 125. In es-

Table II. Elevation of macrophage CSF (>2.5 ng/ml) in women with nonovarian carcinoma

	Eleva	ited	Many (CD	.*
Primary site	No.	. %	$Mean \pm SD = (ng/ml)$	Range
Breast carcinoma	10/54	· 18	1.38 ± 1.19	0.01-4.90
Lung carcinoma	2/6	33	2.39 ± 1.15	0.42-3.60
Colon carcinoma	3/8	38	3.06 ± 2.95	1.25-9.99
Endometrial carcinoma	3/6	50	2.60 ± 1.61	0.98-5.30
· Cervical carcinoma	3/7	43	1.98 ± 1.85	0.01-4.60

tablishing the panel for patients with clinically evident disease, several sera had been selected intentionally bècause CA 125 was <35 U/ml. Only 64% of 69 patients with clinical evidence of disease had CA 125 values >35 U/ml. In the same group of sera, elevated macrophage CSF levels (>2.5 ng/ml) had been found in 68%. Significant complementarity was observed between the two markers. Fourteen of 25 ovarian cancer patients (56%) with clinically evident disease and normal levels of CA 125 (<35 U/ml) had elevated macrophage CSF (>2.5 ng/ml). Sera were available from 37 patients in whom residual disease had been found at surgical surveillance procedures. CA 125 was >35 U/ml in 9 of these patients, (24%) and macrophage CSF was at least 2.5 ng/ml in 15 (40%). Among the 29 patients with serum CA 125 <35 U/ml before positive surgical surveillance procedures, 9 (31%) had at least 2.5 ng/ml macrophage CSF. We have compared macrophage CSF levels in patients with clinical evidence of disease with macrophage CSF levels in patients with disease detected at secondlook surgery to determine the possible effect of tumor burden on the macrophage CSF level. Patients with clinical evidence of disease (mean macrophage CSF level, 3.04 ± 1.83 ng/ml) had significantly higher macrophage CSF levels (p < 0.043, Student t test) than did patients whose disease was detected at second-look surgery (mean macrophage CSF level, $2.29 \pm 1.33 \text{ ng/ml}$).

Elevation of macrophage CSF in nonovarian carcinomas. Macrophage CSF levels were elevated in a variety of nonovarian carcinomas (Table II). A fraction of patients with endometrial (50%), cervical (43%), lung (33%), and colon (38%) carcinomas had at least 2.5 ng/ml macrophage CSF in sera. Elevated levels of macrophage CSF were observed in 10 of 54 patients (18%) with clinically evident breast cancer but in none of 8 patients without evidence of disease recurrence.

Comment

In the absence of malignancy macrophage CSF is produced constitutively by a variety of cells that include fibroblasts and endothelial, epithelial, and placental cells. 8, 18, 19 Certain functionally differentiated cells such as monocytes do not secrete detectable quantities of macrophage CSF unless they are stimulated with phorbol esters or with bacterial lipopolysaccharides. 20, 22 Nor-

mal serum contains some basal amount of colony-stimulating factor.23 A recent report by Shadle et al.24 indicated that sera from a limited number of subjects contained a mean macrophage CSF level of 1.5 ng/ml. The steady-state level of macrophage CSF in serum is governed by the rates of production and clearance. Although the rate and compartments involved in the clearance of macrophage CSF have not been completely resolved, Kupffer's cells and alveolar macrophages may contribute to the removal of macrophage CSF from the circulation by means of specific receptors.25 Macrophage CSF has a very short half-life, and injection of radioiodinated macrophage CSF is quickly cleared from the circulation within 15 minutes. The markedly elevated levels of macrophage CSF observed in patients with renal failure in this study suggest that the renal clearance or metabolism of macrophage CSF deserves further evaluation. Alternatively, processes associated with dialysis or end-stage renal disease might stimulate the production of macrophage CSF.

Elevated serum levels of macrophage CSF were observed in 68% of patients with epithelial ovarian cancer and in a smaller fraction of patients with several other types of carcinoma. The range of concentrations for the marker was relatively narrow, the mean levels in ovarian cancer patients exceeded those in apparently healthy controls by 2.6-fold. Moreover, comparable elevations of macrophage CSF were observed in 31% of patients with benign conditions, particularly renal disease, acute infection, pulmonary disease, and autoimmune disease. In spite of these potential limitations, significant complementarity was observed with CA 125, a high-molecular-weight glycoprotein that is expressed by epithelial ovarian cancers.26, 27 CA 125 has been extensively studied as a marker for monitoring ovarian cancers.28 An immunoradiometric assay that quantitates the antigen can detect from 1.4 to >10,000 U/ml of CA 125 in serum and body fluids. Approximately 99% of apparently healthy blood bank donors will have <35 U/ml of the antigen in serum, although higher values of CA 125 were found in about 6% of individuals with benign diseaes.17 Elevated levels of CA 125 are found in approximately 80% of patients with clinically evident epithelial ovarian cancer, but the remainder of patients with advanced disease have <35 U/ml of the antigen.

At the time of surgical surveillance procedures, an elevated CA 125 level (>35 U/ml) has a positive predictive value of 96%.28 In practice, however, only one patient in five will have an elevated CA 125 level, and in those ovarian cancer patients with serum CA 125 levels <35 U/ml residual disease is found at laparotomy in 40% to 60%. Use of multiple serum markers in combination might improve the sensitivity of CA 125 assays. Among ovarian cancer patients with CA 125 <35 U/ml, macrophage CSF could be detected in 56% of those with clinically evident disease. Elevated macrophage CSF was also detected in sera from 31% of patients without clinically evident disease but in whom occult disease was found at surgical surveillance procedures. Clearly, macrophage CSF deserves further evaluation as a marker for monitoring ovarian cancer patients. The limited specificity of macrophage CSF might be offset by consideration of the clinical status of the patient, as well as by the use of multiple serum markers. Recent studies suggest that the concomitant elevation of CA 125 and TAG 72 or CA 15-3 can provide a more specific test for ovarian cancer than does evaluation of the CA 125 level alone in distinguishing malignant from benign pelvic masses.29,30 After patients with an elevated CA 125 or macrophage CSF in their sera are identified, a concomitant elevation of TAG 72, CA 15-3, or other markers could be sought.

Additional studies are necessary to understand the biologic significance of macrophage CSF in epithelial ovarian cancer. Because some ovarian cancer cells express fms, the receptor for macrophage CSF, autocrine stimulation of ovarian tumor cell growth or function might occur.³¹ Alternatively, macrophages attracted by macrophage CSF to the tumor site could provide paracrine stimulation of tumor growth. Macrophage CSF can act as a chemotactic factor for macrophages³² and may be responsible for the increased number of phagocytic cells observed within some ovarian cancers. Interleukin-1, tumor necrosis factor, and interleukin-6 are produced by macrophages and can stimulate the growth of ovarian cancer cells in culture (unpublished observations). Whether this is important in vivo remains to be determined.

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Postpartum preeclampsia-induced shock and death: A report of three cases

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Three patients with preeclampsia died as a result of prolonged postpartum hypotension that was unrelated to blood loss. Autopsy failed to reveal a cause of death. The sudden onset of hypotensive shock within 24 hours of delivery occurred in all patients, with coexistent hyponatremia present in the two patients in whom it was evaluated. These three maternal deaths appear to have occurred as a result of the previously described entity of postpartum preeclamptic shock. (AM J OBSTET GYNECOL 1991;165:1362-8.)

Key words: Preeclampsia, preeclamptic shock

In 1885 Ballantyne and Buchanan first noted that in some patients with preeclampsia "... after the completion of labour there is a great tendency to complete collapse, and that this unless checked will go on till death closes the scene." Occasional references to

"shocklike collapse" in patients with postpartum preeclampsia have appeared in the medical literature. Twenty-six years later in the United States, Bailey² reported a maternal death 2 hours post partum in a patient with eclampsia whose systolic blood pressure fell from 205 mm Hg just before birth to 100 mm Hg 27 minutes after rapid delivery and after receiving the antihypertensive *Veratrum viride*. Blood loss was reported as minimal. In 1923 Schwarz³ reported a 38.5% mortality rate for patients in whom vascular collapse occurred with preeclampsia. Driscoll¹ considered that "the most disastrous type of shock has been observed in nephritic toxemia," and he hypothesized that the

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cause of postpartum preeclamptic shock involved "... changes in the chemical constituents of blood"

On the basis of a 5-year review of 1018 patients with preeclampsia at the Chicago Lying-In Hospital in 1936, Adair et al.5 noted that patients with preeclampsia often exhibited a sudden postdelivery reduction in blood pressure, then a secondary rise and a rapid fall to values lower than the initial nadir. Twenty-six patients demonstrated a drop in systolic blood pressure of ≤70 mm Hg, 16 also with "profound clinical manifestations of shock." One patient died 2 hours post partum. These cases were attributed to "vascular collapse" caused by "... altered fluid exchange between extravascular and intravascular fluids."5 Dieckmann6 noted that in such cases the hemoglobin concentration was normal, blood loss was negligible, and autopsy findings did not reveal the cause of vascular collapse.

Tatum⁷ contributed eight additional case reports in 1956 and in a subsequent article8 compared the vascular collapse associated with severe preeclampsia-eclampsia with the vascular collapse noted with acute adrenal insufficiency often complicated by low serum concentrations of sodium and chloride. One patient in Tatum's study had a serum sodium level of 112.9 mEq/L. On average, these patients with preeclampsia had mean serum sodium concentrations of 125.9 mEq/L within 2 hours of delivery; the onset of hypotension varied from 15 minutes to 24 hours after delivery. Average blood pressure during collapse was 97/57 mm Hg. All patients were treated with hypertonic saline infusions (at unspecified rates); they recovered without the characteristic second fall in blood pressure levels.8 Interestingly, a decrease in the administration rate of sodium infusion resulted in a rapid decline in blood pressure to hypotensive levels.

A recent reference to the entity of postpartum preeclamptic shock was found only in one textbook,9 and there appear to have been no further case reports. Indeed, references to this syndrome have been deleted from recent texts because there has been a general reduction in maternal mortality among patients with hypertension complicating pregnancy. The dearth of recent reference to preeclampsia may have led to a lack of appreciation of this rare entity.

The purpose of this article is to document three recent fatal cases of postpartum preeclamptic shock and to discuss the diagnostic criteria and presumed pathogenesis of this complication. In two of the three cases litigation led to the correct diagnosis after plaintiffs' attorneys sought to use below-standard-of-care explanations for the then-unexplained deaths.

Case reports

Case 1. A 34-year-old gravida 2, para 1 woman was admitted at 34 weeks' gestation with an initial blood pressure of 160/120 mm Hg. Conservative manage- ... ment led to rapid stabilization and a continuation of pregnancy for 10 days. At that point the fetal biophysical profile began to deteriorate and delivery by cesarean section was elected after the patient was stabilized with magnesium sulfate. During the 24 hours before delivery blood pressure readings varied between 156/100 and 160/106 mm Hg. The abdominal delivery was uneventful, yielding a viable female infant. Blood loss was normal. The patient's blood pressure immediately after delivery was 160/100 mm Hg. Postoperative medications included intravenous magnesium sulfate (1 gm/hr), phenobarbital 100 mg intramuscularly every 8 hours, hydralazine 10 mg for blood pressure >160/100 mm Hg, meperidine 100 mg every 4 hours as needed for pain, and promethazine 25 mg every 4 hours as needed for nausea.

Hydralazine was administered 41/2 hours after delivery when blood pressure increased to 189/116 mm Hg. The rate of intravenous fluid administration was >100ml/hr, and urine output averaged >25 ml/hour. Hematocrit was 35%, and platelet and liver enzyme counts were normal. Nine hours after delivery, the patient was fully awake and conversing with the nursing staff. Thirteen hours after delivery blood pressure was 143/100 mm Hg, and 100 mg of meperidine was administered intramuscularly for pain relief. Fourteen and one-half hours after delivery blood pressure was 90/46 mm Hg, reflexes were 2+, and urine was trace for protein and 2+ for blood. Urine output was adequate. The maternal pulse increased from 100 beats/min 11 hours after delivery to 153 beats/min 14 hours after delivery. At 14% hours after delivery blood pressure decreased to 78/49 mm Hg and pulse decreased to 133 beats/min. The patient responded to painful stimuli only. After aggressive intravenous resuscitation the patient's blood pressure 15 hours after delivery was 114/70 mm Hg.

One hour later the patient's blood pressure precipitately decreased to 78/49 mm Hg, with a pulse of 133 beats/min and respirations 12/min. Two minutes later, blood pressure decreased further to 62/24 mm Hg; the patient remained unresponsive in spite of prolonged efforts to resuscitate her. Serum electrolyte concentrations were not obtained before the patient's death.

An autopsy failed to reveal any anatomic cause for the patient's sudden deterioration and death. Focal edema and small alveolar hemorrhages were found in the lungs. The liver also demonstrated mild focal edema. These changes were attributed to hypotension. Because of the short interval between the severe hypotensive episodes and death, changes were not evident in the brain and no evidence of swelling or herniation was observed. Evidence of disseminated intravascular coagulation was not found. Table I and the Appendix. show clinical and autopsy findings.

Case 2. A 23-year-old primigravid woman was found at her 36-week prenatal visit to have blood pressure of 154/94 mm Hg and nondependent edema; urine reaction for protein was 1+. In spite of bed rest, 10 days later the patient had blood pressure of 140/92 mm Hg and worsening edema; urine reaction for protein was 2+. Spontaneous labor ensued 2 days later, with blood

Table I. Clinical findings

	Case 1	Case 2	Case 3
Age (yr)	34	23	18
Gravida, para	G2, P1	G1, P0	G1, P0
Gestation at time preeclamp- sia was diagnosed (wk)	34	36	36
Findings at time preeclampsia was diagnosed			
Blood pressure (mm Hg)	160/120	. 154/94	
Proteinuria	NA	2+	3+
Edema	NA	Yes	Yes
Rate of infusion of magnesium sulfate before delivery (gm/hr)	1	1	1-2
Method of delivery	Cesarean section	Vaginal	Vaginal
Blood pressure after delivery (mm Hg)	160/100	160/105	ŇA
Initial decrease in blood pres- sure (hr after delivery)	14.5	Immediately and 12	9.5
Serum sodium level (mEq/L)	NA	125	122
Liver enzymes	NA	Normal	Normal
Outcome '	Death	Death	Death

NA, Not available.

pressure 170/108 mm Hg and moderate hyperreflexia; urine reaction for protein was 4+. Magnesium sulfate at 1 gm/hr was infused intravenously. After a 6½-hour labor course, a viable infant was delivered. After the placenta was delivered the patient's blood pressure decreased precipitately from 160/105 to 60 mm Hg/palpable. Simultaneously, the patient's pulse rose to 130 beats/min.

The combination of vigorous cardiorespiratory resuscitation, fluid administration, and dopamine infusion caused a rise in blood pressure to 120/70 mm Hg over the next hour. However, a second decrease in blood pressure to 66 mm Hg/palpable and an increase in pulse to 146 beats/min occurred 30 minutes later. The patient received 500 ml of normal saline solution and 600 ml of 5% dextrose in normal saline solution for resuscitation. Soon thereafter the patient regained consciousness but was unable to move the extremities on her right side. A serum sodium level obtained 15 minutes after the initial hypotensive episode was 125 mEq/L.

Approximately 12 hours post partum the patient became hypotensive, with a 100 mm Hg decrease in systolic pressure within 25 minutes. Resuscitation efforts with hypertonic saline infusions did not restore blood pressure to adequate levels for 1½ hours. A sustained decrease in systolic pressure occurred over a prolonged interval. Normotensive levels were attained only after the serum sodium concentration was normalized. However, the patient died because of ischemic complications from the two prolonged hypotensive episodes.

Autopsy revealed extensive ischemic damage to the small intestine, lungs, kidneys, and brain. Infection had spread throughout the body as a result of bacterial infiltration through ischemic intestinal walls. Death ultimately resulted from adult respiratory distress syndrome. Examination of the brain was refused, although computed tomographic scan had revealed ischemic watershed infarctions (Appendix).

Case 3. An 18-year-old primigravid woman at 36 weeks' gestation had no prenatal complications until 1 week before admission. At admission she had blood pressure of 140/90 mm Hg and trace nondependent edema; urine reaction for protein was 3+. In spite of bed rest, 3 days later she had blood pressure of 140/95 mm Hg and worsening edema; urine reaction for protein was 2+. Induction of labor was undertaken in association with intravenously administered magnesium sulfate at 1 to 2 gm/hr. Urinary output remained >25 ml/hr. Serum electrolyte levels were normal, including a serum sodium level of 137 mEq/L. After only a 51/2-hour first and second stage of labor a 2370 gm male infant with Apgar scores of 9 and 10 at 1 and 5 minutes, respectively, was delivered with low forceps. In the postpartum recovery area the patient continued receiving magnesium sulfate at 2 gm/hr. Urinary output initially averaged 70 ml/hr, and vaginal bleeding was not excessive. Immediate postpartum hematocrit was 36%, hemoglobin concentration was 12 gm/dl, and platelet counts and liver enzyme activity were normal. Nine and one-half hours after delivery the patient had a precipitous drop in blood pressure to 60/40 mm Hg over a 15-minute period in spite of having previously averaged 140/92 mm Hg. The patient became combative and was thought to have seizure activity. A 4 gm bolus of magnesium sulfate was infused immediately, and the seizure activity subsided. Blood drawn at the time of seizure revealed a serum magnesium level of 4.8 mg/dl and normal liver function studies; electrolytes were normal with the exception of the serum sodium level, which was 122 mEq/L. The patient had received 1.8 L of 5% dextrose in water and 0.9 L of 0.5N saline solution during labor.

After anticonvulsive and hypotensive therapy, the patient's blood pressure and pulse increased to 120/70 mm Hg and 96 beats/min, respectively, during a 45-minute interval. Infusion of normal saline solution was continued; however, 1½ hours later a second drop in

blood pressure to 40 mm Hg/palpable was noted, and the patient became unresponsive. Multiple efforts at resuscitation were unsuccessful, and the patient was pronounced dead approximately 12 hours after delivery. Permission for autopsy was refused.

None of the three patients had a history of renal disease or hypertension. Serum creatinine concentrations as a reflection of renal status were within normal limits during the prenatal period. In no instance was excessive (>2 L/24 hr) hypotonic intravenous fluid administered, nor were fluids severely restricted. Serum potassium and glucose values obtained around the time of the hypotensive episodes (cases 2 and 3) were within normal limits.

Comment

Preeclampsia, especially in its severe form, is characterized as a state of increased cardiac output, vascular reactivity, and peripheral vascular resistance.10 A redistribution of intravascular volume into the extravascular space occurs with resultant decreased plasma volume but normal pulmonary capillary wedge and central venous pressures. A derangement of cellular sodium homeostasis is considered to play a considerable role in the pathophysiologic development of preeclampsia." Unfortunately, detailed sequential antepartum electrolyte studies in women with a tendency to develop preeclampsia do not exist, although the hemoconcentration usually observed in these subjects is associated with normal serum sodium concentration in most patients. 12

The subjects described in this report and those sporadically described since 1885 suggest that there is a small subset of women with preeclampsia whose postpartum mobilization of fluid and sodium from the extravascular space is delayed. Low serum sodium concentrations develop in association with hypotensive episodes; such patients are unlikely to survive unless there is rapid infusion of a hypertonic saline solution as proposed by Tatum.7 It is unknown why a small number of patients with severe preeclampsia fail to mobilize and maintain sufficient serum sodium concentrations during the early postpartum period, thereby precipitating vascular collapse and shock. The reason could be related to adrenal corticosteroid production. Tatum theorized that after delivery there may ensue an acute period of adrenal cortical insufficiency that disrupts the process of rapid extravascular-intravascular sodium movement and thereby precipitates vascular insufficiency and collapse. Studies in rabbits suggest that cerebral adaptation to hyponatremia is less efficient in females than in males.13 Unusually increased serum progesterone concentrations may participate in this differential response between sexes and among these patients because it has been shown that progesterone inhibits sodium potassium adenosine triphosphatase (ATPase) activity.14 Although most patients with severe preeclampsia have lowered plasma volume and slow but modest reductions in blood pressure post

partum, there appear to be a small number whose aberrant fluid mobilization precipitates a severe hypotensive

Review of the patients described in this report suggests that clinical severity of preeclampsia and method of delivery are unrelated to the observed vasomotor collapse. Furthermore, the hypotensive episodes associated with this syndrome are not alleviated by circulatory volume replacement alone. Thus the explanation could lie in the hemodynamic role of sodium in the vasoconstriction process. Previous authors have speculated that an aberrrant relationship between adrenal corticosteroids and low serum sodium levels could be a triggering mechanism.7.15

Caution is advised, however, with the use of hypertonic saline solution to correct symptomatic hyponatremia. Overly aggressive elevation of serum sodium concentration by >25 mmol/L in 24 hours can result in diffuse cerebral demyelinating lesions.16 Some authors now recommend that in symptomatic short-term hyponatremia the serum sodium concentration be increased rapidly only by 10%, with additional increases being achieved very slowly by water restriction. 17,18 Mortality associated with serum sodium concentrations >149 or 160 mmol/L is 40% and 60%, respectively, in elderly hospitalized patients.19 Recommendations for clinical management of symptomatic hyponatremia in gravida women with preeclampsia and vascular collapse are problematic because there exist no published clinical trials of comparative therapy. Because red blood cell Na+/K+-ATPase activity in women who are hypertensive and pregnant has been reported to be reduced in comparison to gravid women who are normotensive,20 and because efficient Na+/K+-ATPase activity in the brain is required to maintain sodium homeostasis in that organ,13 abnormal membrane cation transport may play a role in the cause of postpartum preeclamptic shock.

Autopsies of two of the three patients revealed ischemic damage to major organs as a result of acute and prolonged hypotension (Appendix). Microscopic examination of the renal glomeruli of both autopsied patients on whom autopsies were performed showed microscopic changes consistent with preeclampsia. No patient had significant hemorrhage resulting in loss of blood volume. The autopsy of the patient in case 1 revealed "no apparent anatomic cause of death." Cause of death was also indeterminate in case 2, with the principal anatomic diagnosis listed as "eclampsia, septicemia, and cerebral infarctions."

Two of the three cases were undiagnosed as to cause of death and resulted in malpractice litigation. Both the treating physicians (and thereby subsequent defendants) and the expert witnesses engaged by plaintiffs were unaware of the existence of postpartum preeclamptic shock as a complication, perhaps because of a dearth of case reports in the last 30 years and because

of the rarity of the malady. In case 1 the plaintiff claimed that the hypotensive episode occurred as a result of postoperative medications. In case 2 the bad result and organ injury with disseminated intravascular coagulation were alleged to be caused by delay of treatment with pressor agents. In neither case did the objective evidence justify such conclusions.

In summary, severe postpartum hypotension in patients with preeclampsia can ocur and result in maternal death. A high index of suspicion will allow the obstetrician to measure serum sodium, osmolarity, and perhaps cortisol levels in patients having such symptoms. Aggressive supportive measures that include the infusion of hypertonic saline solution may be helpful. Although the cause of this syndrome remains unclear, these recent patient experiences demonstrate the current necessity for obstetricians to be aware of the possibilities for such complications among patients with preeclampsia.

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Appendix

Autopsy findings: Case 1

Cardiovascular system

The heart weighs 300 mg. There is no evidence of recent or old myocardial infarction. The coronary ostia on both sides are patent and the coronary arteries show no atherosclerotic thickening. The rest of the aorta and major arteries show a minimal degree of atherosclerosis. The superior vena cava and its tributaries are within normal limits, with no evidence of thrombus or embolus.

Respiratory system

The left lung weighs 340 gm and the right lung weighs 460 gm. The surfaces of the lungs are soft and pinkish gray. The dependent areas of both lungs are red-purple. The trachea and bronchi are normal. The pulmonary vasculature is normally developed, with no evidence of thrombi or emboli. Significant pulmonary edema is noted in both lungs, and a moderate degree of parenchymal hemorrhage is noted in both lungs.

Microscopic examination
Lungs: Focal atelectasis.

Genitourinary system

Kidneys: The kidneys are large, weighing 250 gm and 260 gm for left and right, respectively. The capsules of the kidneys are removed with ease and reveal a smooth reddish tan surface. Cut sections show normal corticomedullary ratio and very prominent congested medullary portion. The pelvicalyceal system is normal. Focal and segmental mesangial proliferation of the glomeruli is seen.

Appendix-Cont'd

Autopsy findings: Case 1-cont'd

Uterus: The uterus is enlarged and firm; in the uterine cavity a few blood clots are present with roughening of the mucosal surface at the site of placental implantation. The cervix is soft, congested, and dilated.

Ovaries: Normal.

Central nervous system

Brain: The calvarium shows diffuse hemorrhagic discoloration in the subperiosteal region. The dural surfaces are smooth, grayish white, and glistening. The brain weighs 1350 gm and is soft. External examination of the formalin-fixed brain shows symmetric hemispheres with normal gyrational pattern; no evidence of swelling or herniation is present. The circle of Willis shows a normal vascular distribution, and there is no evidence of atherosclerosis or other vascular abnormalities. The leptomeninges are smooth and translucent. The dural segment that is available shows pronounced ossification in several areas close to the sagittal sinus. Multiple coronal sections fail to show any abnormalities. The ventricular system is of usual size and shape. The brain is quite pale, but there are no focal lesions in the cerebral hemispheres, brain stem, cerebellum, or spinal cord.

Autopsy findings: Case 2

Cardiovascular system

The heart weighs 380 gm. There is minimal atherosclerosis of the right coronary artery. The coronary arteries are collapsed. There are minimal atherosclerotic streaks in the abdominal aorta. The vena cava and its tributaries are patent.

Respiratory system

The right lung weighs 750 gm and the left lung weighs 660 gm. The pleural surfaces are smooth and glistening, with multiple areas of hemorrhage. The bronchial mucosa is granular and green-gray. The segmental vessels of the right upper lung appear occluded by recent thrombus. There are multiple hemorrhagic infarcts in the right upper lobe. Numerous areas of the hemorrhage and congestion are present. The rest of the parenchyma shows uniform consolidation. The alveolar spaces seem to be enlarged. The pulmonary artery and veins are patent.

Microscopic examination

Main bronchus: There is almost complete necrosis of the epithelium. Abundant fungal colonies are present. There is also extensive submucosal hemorrhage.

Lungs: Multiple sections of both lungs show extensive areas of recent hemorrhage without destruction of the lung parenchyma. There are also numerous areas of necrosis with abundant polymorphonuclear cell infiltrate. Airways are filled with mucus, red blood cells, and polymorphonuclear cells. Multiple fungal colonies are present in the mucosa of the bronchi. In many areas alveolar spaces are filled with pink homogeneous, proteinaceous material and hyaline membranes. Fungal colonies are also seen in the parenchyma. On the pleural surface there is fibrin. No epithelial squames are seen. Immunohistochemical stain with the antibody against keratin did not reveal any keratin positivity in the blood vessels. Fibrin thrombi in the blood vessels are seen. There are numerous megakaryocytes in the alveolar space.

Genitourinary system

Kidney: The left kidney weighs 130 gm and the right weighs 135 gm. They are normal in shape. The glomeruli appear slightly swollen and pale. The capsule is not adherent. On removal of the capsule, the outer surface is smooth with fetal lobulations. The corticomedullary junction is normal. The calyces and pelves are normally oriented. There are multiple petechial hemorrhages on the pelvic mucosa. There is extensive tubular necrosis. Multiple granular casts are seen in the tubules. Numerous autolyzed epithelial cells are seen in the tubes. Glomeruli show widening of the glomerular basement membrane. Occasionally endothelial swelling is seen. The glomeruli appear slightly swollen.

Uterus: The uterus is markedly enlarged. It weighs 1200 gm and measures $22 \times 18 \times 9$ cm. The endometrium is hemorrhagic, and multiple irregular hemorrhagic tightly

Appendix—Cont'd

Autopsy findings: Case 2-cont'd

adherent membranes are seen. The myometrium and cervix are hemorrhagic. There are abundant partially necrotic membranes. Gram's stain reveals numerous positive cocci. Bacterial colonies are also present in the myometrium.

Ovaries: Two ovaries are present. These are enlarged with hemorrhagic surface. The left ovary measures $3.5 \times 1.8 \times 1.9$ cm and the right measures $3.7 \times 2.2 \times 1.9$ cm. The cut surface shows multiple cystic spaces measuring up to 0.5 cm in diameter.

Central nervous system

Brain: Not done.

Renal agenesis in association with malformation of the female genital tract

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Four cases of renal agenesis with an ipsilateral blind vagina and two cases with agenesis of all organs derived from one urogenital ridge are presented. These cases confirm the association of renal agenesis with these genital malformations and support the hypothesis that the embryologic development of the vagina is derived from the fused mesonephric ducts. (AM J OBSTET GYNECOL 1991;165:1368-70.)

Key words: Renal agenesis, genital malformations, embryology of the vagina

Malformations of the female genital tract can be associated with various urinary tract anomalies, but some of these anomalies are also observed in women without genital malformations. However, in cases of renal agenesis there is always an associated genital tract malformation. Detailed analysis of these malformations has allowed us to propose the hypothesis that the embryologic development of the vagina is derived from the wolffian ducts. We report six new cases of renal agenesis, four with ipsilateral blind vaginas and müllerian duplication and two with agenesis or severe hypoplasia of all organs derived from one urogenital ridge and skeletal anomalies. Table I summarizes symptoms and

some findings of the six case reports. Fig. 1 shows the schema of the urogenital tracts in these cases.

Comment

In general, all cases of unilateral blind vagina are associated with renal agenesis (or hypoplasia if there is an ectopic ureter opening into the blind vagina). On the other hand, all cases of renal agenesis are associated with blind vagina (or Gartner's pseudocyst, which is actually an atretic blind vagina) and usually müllerian duplication caused by the failure of the inductor function of the mesonephric duct.² The exceptions to the rule of renal agenesis—blind vagina are agenesis or severe hypoplasia of all organs derived from the urogenital ridge (cases 5 and 6) and some cases with partial reabsorption of the intervaginal septum, in these cases this septum is not typically present in the inferior third of the vagina.¹

Renal agenesis—blind vagina association can be caused by a lesion of the mesonephric duct (with the junction of the müllerian tubercle, the origin of the vagina) which causes there to be no opening in the

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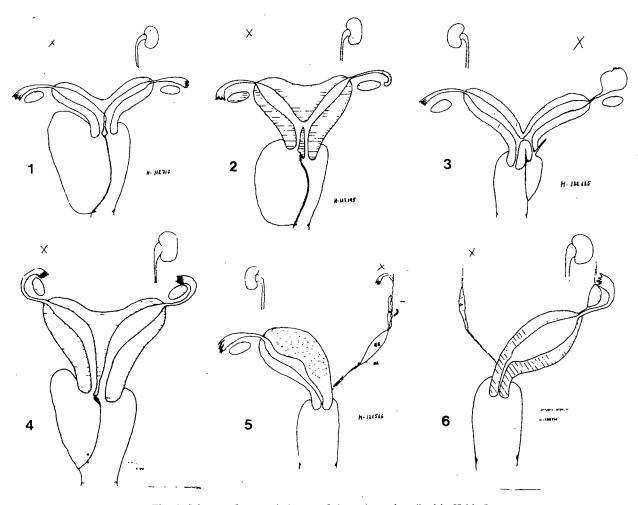


Fig. 1. Schema of urogenital tract of six patients described in Table I.

Table I. Summary of case reports

Case No.	Age (yr)	Age at menarche (yr)	Symptoms	Renal agenesis	Epithelium lining blind vagina	Skeletal anomalies
1	17	10	Abdominal pain, sudden discharge of brown, malodorous fluid at end of menstruation	Right	Cubic, monolayer, and eroded, suggesting müllerian epi- thelium	
2	18	12	Increasing dysmenor- rhea from menarche with persistent post- menstrual blood, mal- odorous discharge	Right	Eroded with chronic inflammation and focal squamous metaplasia without remnant of original epithelium	_
3	29	. 13	Large quantity of brown fluid expelled at age 16 years; cesarean sec- tion for breech pre- sentation at age 25 years; sudden puru- lent intermenstrual discharge	Left	Squamous with strong inflammatory component; inside septum were wolffian and müllerian remnants	~
4	18		Dysmenorrhea from menarche; dark, bloody postmenstrual discharge for several days; malodorous dis- charge from 6 months earlier (sexual activity)	Right	Squamous and eroded epithelium; mülle- rian remnants in- side intervaginal septum; glands lined by cubic cells	Dorsal scoliosis

Table I-Cont'd

Case No.	Age (yr)	Age at menarche (yr)	Symptoms	Renal agenesis	Epithelium lining blind vagina	Skeletal anomalies
5	43	13	Menorrhagia; large uterine leiomyomas; severe hypoplasia or agenesis of all organs derived from left uro- genital ridge	Left	_	Dorsolumbar sco- liosis
6	31	12	Cesarean section for breech presentation	Right	-	Three fused lumbar vertebrae (L-2–L- 4), severe cervico- dorsal scoliosis

urogenital sinus and no upward migration of the ureteral bud from this opening toward the metanephric blastema, which normally permits the development of the normal kidney.¹

These cases support our hypothesis that the embryology of the vagina is derived from the fused mesonephric ducts. Müller's tubercle (no ducts) would probably be required for adequate tunneling and lining, first by columnar or cubic epithelia and then epidermization. Thus the epithelium lining the blind vagina is generally of the müllerian type, but when there is transseptal communication or inflammatory alterations or when the septum is removed, the lining converts to squamous, stratified epithelia or shows squamous metaplasia. Wolffian and müllerian remnants can be found in the septum (cases 3 and 4).

Finally we must emphasize that when the symptoms of dysmenorrhea and postmenstrual bloody, malodorous discharges occur these malformations, especially the unilateral blind vagina, should be sought. Patients are usually first seen with either primary or secondary communicating uteri; through this communication a persistent infected hematocolpos can result.

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The association between fetal karyotype and mean corpuscular volume

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To determine whether elevated fetal mean corpuscular volume is characteristic of a chromosome abnormality or fetal disease, 22 fetuses with chromosome abnormalities, 31 with uteroplacental insufficiency, 50 undergoing their first cordocentesis for hemolytic disease, and 50 control fetuses were identified. Chromosomally abnormal fetuses had a significantly higher mean corpuscular volume than the control fetuses. Among fetuses with chromosome abnormalities, the mean corpuscular volume for trisomic or triploid fetuses was significantly higher than for fetuses with other chromosome abnormalities. An elevated mean corpuscular volume was also associated with uteroplacental insufficiency, intrauterine growth retardation, and hemolytic disease. It correlated significantly with gestational age (p < 0.002 in all cases) in all groups except trisomy or triploidy. In addition, it correlated with hematocrit in the hemolytic disease group (r = -0.60, p < 0.0001) and with Po₂ in fetuses with intrauterine growth retardation (r = -0.43, p = 0.005) from all causes including uteroplacental insufficiency. Trisomic or triploid fetuses showed no such relationships and therefore appear to have escaped the normal control mechanisms for erythropoiesis. One in 12 fetuses with an elevated mean corpuscular volume had trisomy or triploidy, whereas no fetus with trisomy or triploidy had a normal mean corpuscular volume. Thus an unexpectedly elevated fetal mean corpuscular volume in a patient undergoing cordocentesis for reasons other than evaluation of fetal chromosomes would appear to warrant further karyotypic analysis. (AM J OBSTET GYNECOL 1991;165:1371-6.)

Key words: Aneuploidy, fetus, karyotype, mean corpuscular volume

In 1986 we observed a very high mean corpuscular volume, 175.0 fl, in a blood sample obtained from a fetus ultimately shown to have trisomy 21. If a high mean corpuscular volume were characteristic of chromosome abnormalities, it could be used as an indication for karyotypic evaluation. Only one report in the literature has previously addressed this question. The results of cordocenteses performed between 1985 and 1990 in the University of Iowa Fetal Diagnosis and Treatment Unit were reviewed to determine whether a high mean corpuscular volume was typical of fetuses with a chromosome abnormality and whether other fetal diseases were associated with an elevated fetal mean corpuscular volume.

Material and methods

The study group was drawn from >300 fetuses evaluated at the University of Iowa Fetal Diagnosis and Treatment Unit for a variety of clinical indications.²

The mean corpuscular volume was determined in 267 as part of routine testing. Final diagnoses were based on antenatal and postnatal evaluation.

The technique for fetal blood sampling has previously been described in detail.² The vessel punctured is identified by (1) ultrasonographic image, (2) the direction of turbulent flow after injection of saline solution or pancuronium, (3) the presence or absence of a pulsatile waveform on a blood pressure monitor,³ and (4) pulse-gated Doppler (5 MHz transducer, GE 3600, General Electric, Rancho Mirage, Calif.) interrogation. A combination of these four techniques eliminates any question regarding the identity of the punctured vessel.

A heparinized specimen for fetal blood gas measurement was immediately analyzed (Instrumentation Laboratories, 1312 Blood Gas Manager, Lexington, Mass.). The pH, PCo₂ (millimeters to mercury) and Po₂ (millimeters of mercury) were measured directly, assuming a temperature of 37° C. A complete blood cell count was performed with a blood sample in ethylenediaminetetraacetate on automated equipment (Technicon, Tarrytown, N.Y.) and confirmed manually when necessary.

Previously published norms for umbilical venous PO₂ were recalculated with a larger sample size and gestational age epochs altered for the present study (data not shown). Norms for the mean corpuscular volume

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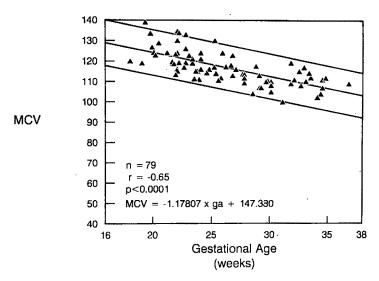


Fig. 1. Normal mean corpuscular volume (MCV) in femtoliters versus gestational age in weeks including 95% prediction intervals.

from 18 through 34 weeks were derived from 79 blood samplings on 50 control fetuses. These fetuses underwent diagnostic cordocentesis and were ultimately shown to be normal or unaffected by the disease for which they were being tested (i.e., Rh-negative fetuses in Rh-sensitized pregnancies). We defined an elevated mean corpuscular volume as being >95% prediction interval for each gestational age epoch. An abnormal umbilical venous Po₂ was defined as <95% prediction interval for each gestational age epoch.

Gestational age was determined from the earliest available fetal ultrasonographic measurements. Fetuses with intrauterine growth retardation (IUGR) met the following criteria for diagnosis⁴: (1) an antepartum ultrasonographic abdominal circumference measurement <2.5th percentile for gestational age in healthy fetuses delivered at term and (2) postpartum confirmation of birth weight <10th percentile for gestational age by an appropriate table.⁵ A subgroup of fetuses with IUGR associated with uteroplacental insufficiency was identified from within the IUGR group, after elimination of fetuses with a chromosome abnormality or documented infection.

All fetuses with abnormal karyotypes were included in the chromosome abnormality group. Although several fetuses shared this abnormality with a parent, associated fetal structural defects make the existence of microscopic deletions or duplications of chromosome material probable.

The results were presented as the mean \pm SD. Analyses were done with subroutines from Abstat rel 6.02 (Anderson-Bell, Parker, Col.). An independent t test was used to compare the gestational ages and mean corpuscular volume values between groups of fetuses. Cross tabulation with Fisher's exact test was used to

compare the percentage of fetuses with IUGR or an elevated mean corpuscular volume in the various diagnostic groups. Stepwise multiple linear regression was used with mean corpuscular volume as the dependent variable and gestational age, hematocrit, and umbilical venous Po2 as independent variables. An independent variable was added to the regression when it contributed a p < 0.1. Mean (\pm SD) mean corpuscular volume and umbilical venous PO2 values were calculated for the various gestational age epochs, using the 95% prediction intervals, where appropriate. A $p \le 0.05$ was assumed to reflect a significant difference among groups or a significant relationship among variables. Because of multiple comparisons, $p \le 0.01$ was assumed to reflect a significant difference in the multiple linear regression analysis.

Results

Four groups of fetuses were compared: control (n = 50), chromosomally abnormal (n = 22), growth-retarded in association with uteroplacental insufficiency (n = 31), and hemolytic disease (n = 50). The latter two disorders were selected because they have been associated with high mean corpuscular volume values. A second, larger growth-retarded group (n = 63) included all fetuses with available mean corpuscular volume values who fulfilled the criteria for diagnosis of IUGR (all causes).

The mean corpuscular volume values for control fetuses declined with increasing gestational age (Fig. 1). Fetuses with chromosome abnormalities (A) were significantly older than those in the control (C) group (A, 29 ± 6 weeks and C, 24 ± 4 weeks; p = 0.0008). Although the higher mean gestational age of the fetuses with chromosome abnormalities should be associated

Table I. Comparison between fetal groups (mean \pm SD)

	No.	Gestational age (wk)	Mean corpuscular volume (fl)
Control	50	24 ± 4	119 ± 8
Chromosome abnormality	22	$.29 \pm 6*$	$130 \pm 23*$
Uteroplacental insufficiency	31	31 ± 4*	122 ± 14
Hemolytic disease	50	. 26 ± 4*	123 ± 15

^{*}p < 0.05, from control.

Table II. Karyotypes and growth of fetuses with an euploidy and structural rearrangements (n = 22)

Case No.	Karyotype .	IUGR	Gestational age (wk)	Mean corpuscular volume (fl)
Trisomy-tri	ploidy group			
1 '	69,XXY	No	19	. 175
.2	47,XX,+18	No	29.6	114*
3 .	47,XY, +18	Yes	. 33.6	134*
4	47,XX,+21	Yes	28.7	143*
5	47,XY, +18	Yes	32	120*
6	69,XXY	Yes	. 27	168*
7	47,XX,+21	No	33	118*
8	47,XY, +18	Yes	34	133.6*
. 9	69,XXX	Yes	3 27.5	157*
10	69,XXY	No	20.0	184*
11	47,XY, +18	Yes	30.1	116*
Other chron	nosome abnormalities			
1	46,XX,t(3;4)(q25;p16)mat	Yes.	37.7	113
. 2	46,XX,-15,+t(15;20)(q15;q13.1)	No	31	114
3	46,XY/47,XXY	No	21	141*
4	45,X/46,XX	No	22	128*
5	46,XX,t(6;13)(p25;q22)	· No	33.3 ¹	113*
6	46,XX/47,XX,+21	No	20	125.3*
7	46,XX,-5,+der(5)t(5;?)(p15.1;?)	No	23	115
8	46,XY,inv(10)(p11.2;q21.2)pat	Yes	36.4	94.5
9	46,XX,del(7)(q36.1)	No	22.4	121.6
10	46,XY,inv(11)(p15q13)pat	Yes	33.8	107
11	46,XY,-12,+der(12)t(3;12)(p21;p13)	No	34.0	116*

Mean corpuscular volume elevated for gestational age (outside 95% prediction interval).

with lower mean corpuscular volume values, their mean corpuscular volume values were significantly higher than those of the control fetuses (A, 130 ± 23 fl and C, 119 ± 8 fl; p = 0.0030) (Table I).

Sixty eight percent (43/63) of growth-retarded fetuses and 46% (23/50) of fetuses with hemolytic disease had elevated mean corpuscular volume values for gestational age ($p \le 0.0001$). In the hemolytic disease group and the subgroup of growth-retarded fetuses with uteroplacental insufficiency, the mean mean corpuscular volume values were similar to those of controls, because fetuses in both groups were significantly older than the control fetuses (Table I).

On the basis of prior observations,⁶ the 22 chromosomally abnormal fetuses were subdivided into a group with either autosomal trisomy or triploidy (n = 11) and a group comprising all other chromosome abnormalities (n = 11) (Table II). The mean mean corpuscular volume for the trisomic-triploid fetuses (T) was significantly higher than the mean for fetuses with other (O)

chromosomal abnormalities (T, 142 ± 25 fl and O, 117 ± 12 fl, p = 0.0039). All trisomic or triploid fetuses (11/11) had high mean corpuscular volume values, whereas elevated mean corpuscular volume values were found in only 45% (5/11) of fetuses with other chromosome abnormalities (p < 0.025). The nonsignificant gestational age difference (T, 29 ± 5 weeks and O, 27 ± 7 weeks; p = 0.99) does not explain this phenomenon. Could growth retardation be the cause?

A high mean corpuscular volume did not correlate with growth retardation per se among the chromosomally abnormal fetuses. Although 64% (7/11) of the trisomic-triploid fetuses were growth-retarded compared with 27% (3/11) of the fetuses with other chromosome abnormalities (p=0.08), eight of the 16 karyotypically abnormal fetuses with mean corpuscular volume values that were elevated for gestational age were growth-retarded and eight were not.

Three of the fetuses in the other chromosome abnormality group could not be proved to have an un-

Table III. Elevated mean corpuscular volume—an indication of trisomy or triploidy

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
All fetuses $(n = 267)$, 100	54	8	100
Normally grown fetuses $(n = 204)$	100	57	4	100
Growth retarded fetuses $(n = 63)$	100	43	18	100
Excluding fetuses with hemo	lytic disease			
All fetuses $(n = 217)$	100	. 54	. 10	100
Normally grown fetuses $(n = 154)$	100	58	6	100

balanced defect, because grossly the translocation or inversion was shared with a parent. However, a microscopic duplication or deletion undetectable by present methods cannot be ruled out and seems probable in view of the associated fetal anatomic abnormalities: hydrocephalus with Arnold-Chiari malformation, myelomeningocele, ventricular septal defect, omphalomesenteric band, polymicrogyria, and small colon (case 1); polyhydramnios, severe IUGR, holoprosencephaly, pulmonary hypoplasia, hypoplastic midface, single nares, renal hypoplasia, and ambiguous genitalia (case 8); sirenomelia, sacral meningocele, atrial septal defect, bilateral renal agenesis, anhydramnios, distal tracheoesophageal fistula, and proximal esophageal atresia (case 10). Even if these fetuses are excluded from the O group (O-3), the mean corpuscular volume continues to be significantly higher in the trisomic-triploid fetuses (T, 142 ± 25 fl and O-3, 122 ± 10 fl; p = 0.023). Gestational age differences were not significant (T, 29 ± 5 weeks and O-3, 26 ± 6 weeks; p = 0.14).

Could an elevated mean corpuscular volume be useful as a test for increased risk of trisomy or triploidy? To address this question, fetuses were divided into normally grown and growth-retarded groups, since early onset IUGR is the second leading indication for karyotypic evaluation in our center. The groups were evaluated both before and after exclusion of the fetuses with hemolytic disease (Table III). The sensitivity and negative predictive value of an elevated mean corpuscular volume for trisomy or triploidy were consistently 100%, and specificity ranged from 43% to 58%. The positive predictive value ranged from 4% to 18% and was not substantially improved by exclusion of the hemolytic disease group. No fetuses with hemolytic disease had IUGR or a chromosome abnormality.

The mean corpuscular volume correlated negatively with gestational age (r=-0.51, p<0.0001) and umbilical venous Po₂ (r=-0.14, p=0.046) in all fetuses. Stepwise linear regression was applied to determine which variables had the most impact on the mean cor-

puscular volume (Table IV). The principal determinant of mean corpuscular volume was gestational age in all four groups. Mean corpuscular volume was significantly related to the umbilical venous Po2 in the uteroplacental insufficiency group. These fetuses illustrate the relationship between low Po2, IUGR, and mean corpuscular volume. Fifty-eight percent of fetuses in the study population with an abnormal Po₂ (n = 156)had an elevated mean corpuscular volume for gestational age. When fetuses with IUGR (n = 63) were excluded from the analysis, any significant relationship between mean corpuscular volume and umbilical venous Po₂ in the remaining fetuses was lost (r = -0.003, p = 0.9738). Mean corpuscular volume was significantly related to hematocrit in the hemolytic disease group. In the chromosome abnormality group, mean corpuscular volume was related only to gestational age.

When the chromosome abnormality group was subdivided by diagnosis, the trisomic-triploid fetuses had lost even the relationship between mean corpuscular volume and gestational age. Thus the normal controls of erythropoiesis and ultimately the mean corpuscular volume during fetal life no longer appeared to function in the trisomy and triploidy fetuses.

Comment

Mean corpuscular volume is higher in human fetuses than in adults and decreases with increasing gestational age.^{1, 8-10} This gestational age—dependent decline is illustrated by the control euploid fetuses (Fig. 1). In part the higher fetal mean corpuscular volume is due to an increased concentration of hemoglobin F in fetal red blood cells. Our study demonstrates that an elevated fetal mean corpuscular volume also relates to certain disease processes.

Uteroplacental insufficiency, fetal hemolytic disease, and aneuploidy have been reported to be associated with an elevated mean corpuscular volume. 1, 6, 7 Our findings confirmed these reports. However, we also noted that fetuses with autosomal trisomy or triploidy had significantly higher mean corpuscular volume mea-

Table IV. Correlation of mean corpuscular volume with gestational age, hematocrit, and umbilical venous Po2

Correlation of mean corpuscular volume and other parameters	r	p	Multiple r	p
Control $(n = 50)$				
Gestational age	-0.85	< 0.0001	0.71	< 0.0001
Hematocrit				
Umbilical venous PO ₂	-0.31	0.026		
Uteroplacental insufficiency				
(n=31)				
Gestational age	-0.62	0.0003	0.77	0.0001
Hematocrit				
Umbilical venous PO2	-0.42	0.0054		
Hemolytic disease $(n = 50)$				
Gestational age	-0.46	0.0019	0.79	< 0.0001
Hematocrit	-0.60	< 0.0001		
Umbilical venous PO ₂	-0.25	0.07		
Chromosome abnormality $(n = 22)$				
Gestational age	-0.73	0.01	0.71	0.047
Hematocrit	****	NS	****	0.01.
Umbilical venous PO ₂				
Trisomy-triploidy $(n = 11)$				
Gestational age		NS		
Hematocrit	•			
Umbilical venous PO2				
Other chromosome abnor-				
malities $(n = 11)$				
Gestational age	-1.08	0.0006	0.96	0.0029
Hematocrit	0.45	0.02		
Umbilical venous PO2				

NS, Not significant.

surements than did fetuses with other chromosome abnormalities. It is interesting to note that all of the aneuploid fetuses in both groups (i.e., including those with mosaicism) had elevated mean corpuscular volume values. The elevated mean corpuscular volume in trisomytriploidy was at least partially independent of growth retardation. The lack of a relationship between mean corpuscular volume and gestational age, hemoglobin, and umbilical venous Po2 in fetuses with trisomy or triploidy suggests that their erythropoietic mechanism is not subject to the usual physiologic controls. This raises the possibility of using an elevated mean corpuscular volume as an indication for karyotypic evaluation.

A normal mean corpuscular volume in our study population completely ruled out fetal triploidy or trisomy (negative predictive value, 100%). The sensitivity of an elevated mean corpuscular volume for trisomy or triploidy was 100% compared with 64% sensitivity of the presence of IUGR alone for trisomy or triploidy. Early onset IUGR is the second leading indication for karyotypic evaluation in our center. This high sensitivity of an elevated mean corpuscular volume for trisomy or triploidy suggests that it may identify a higher percentage of these aneuploid fetuses than the diagnosis of IUGR alone. However there are several reasons that a fetus may have an elevated mean corpuscular volume,

as discussed previously. By including or excluding these other groups, the positive predictive value ranged from 4% to 18% (Table III). It was not substantially improved after excluding the fetuses with hemolytic disease. This positive predictive value range is compatible with the yield from screening tests such as high maternal serum α-fetoprotein (3.3%),11 low maternal serum α-fetoprotein (1.1%),12 and amniocentesis because of maternal age >35 years (<0.5%). Within a referral population similar to our study population an elevated mean corpuscular volume could be considered as a potential marker for trisomy and triploidy but not for the diagnosis of other chromosome abnormalities. Because of the relatively small sample and the fact that some of the atypical chromosome rearrangements (e.g., translocations) identified were not associated with an elevated mean corpuscular volume, we do not recommend withholding karyotypic evaluation from fetuses with a normal mean corpuscular volume.

Could the elevated mean corpuscular volume associated with these varied disease processes have a single explanation? One possibility would be decreased fetal blood oxygen content either in association with a decrease in oxygen transport (as in the fetus with uteroplacental insufficiency) or a decrease in oxygen-carrying capacity (as in the anemic fetus). The increase in erythropoiesis in severely anemic fetuses is associated with a shift toward extramedullary erythropoiesis. An increased percentage of hemoglobin F enhances the oxygen-carrying capacity of fetal blood and may occur in response to fetal hypoxia.18 Therefore, in the presence of decreased fetal oxygen content, increased extramedullary erythropoiesis and increased hemoglobin F concentration may be compensatory mechanisms. Each would result in macrocytosis. What of the fetuses with trisomy or triploidy? There was no relationship between mean corpuscular volume and either hematocrit, Po2, or gestational age in these fetuses. Perhaps red blood cell handling of oxygen or hemoglobin F concentration is abnormal in trisomy and triploidy. Identification of the ways in which their control differs from that of euploid fetuses might further define the normal control of erythropoiesis in utero.

In conclusion, the elevated mean corpuscular volume in fetuses with either IUGR, hemolytic disease, or a low Po₂ likely results from a decrease in fetal blood oxygen content. In fetal trisomy and triploidy, the mechanism may be an excess concentration of hemoglobin F or another intrinsic red blood cell abnormality. In fetuses without hemolytic disease, an elevated mean corpuscular volume indicates a fetus at increased risk for trisomy or triploidy and should lead to karyotypic evaluation.

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Cytologic localization of epidermal growth factor and its receptor in developing human placenta varies over the course of pregnancy

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Cytologic localization of epidermal growth factor and its receptor in developing human placenta was analyzed by avidin-biotin immunoperoxidase techniques with a polyclonal antibody to epidermal growth factor and a monoclonal antibody to its receptor. In 4- to 5-week placenta, epidermal growth factor and its receptor were found to be almost exclusively localized to cytotrophoblasts, whereas in 6- to 12-week placentas they were predominantly localized to syncytiotrophoblasts. These findings suggest that both are initially expressed in cytotrophoblasts in very early placenta before 6 weeks' gestation and thereafter expressed in syncytiotrophoblasts in 6- to 12-week placentas. Their simultaneous expression in the cytotrophoblast of 4- to 5-week placentas and in the syncytiotrophoblast of 6- to 12-week placentas implies that epidermal growth factor may act in an autocrine manner in first-trimester placentas. By contrast, in second- and third-trimester placentas, epidermal growth factor was mainly localized to cytotrophoblasts, whereas its receptor was predominantly localized to syncytiotrophoblasts. These findings imply that epidermal growth factor may act in a paracrine fashion in second- and third-trimester placentas. The dynamic change in cytologic localization of epidermal growth factor and epidermal growth factor receptor in developing human placentas may reflect the change in a possible role of epidermal growth factor in the course of fetoplacental development. (Am J Obstet Gynecol 1991;165:1377-82.)

Key words: Placenta, epidermal growth factor, epidermal growth factor receptor, immunohistochemistry

Epidermal growth factor (EGF) is a single-chain polypeptide that is a potent mitogen in vivo and in vitro for a variety of normal and neoplastic cells.^{1, 2} It also influences differentiated cellular functions in the absence of a mitogenic effect^{3, 4} and even inhibits the growth of A431 human carcinoma cells, which contain the highest number of EGF receptors.⁵

In relation to fetoplacental development, EGF has been reported to be linked to the molura to blastocyst transformation,⁶ increased protein synthesis in trophectoderm cells of preimplantation mouse blastocyst,⁷ and successful completion of pregnancy in mice.⁸ In humans EGF has been implicated in the development

of the fetal digestive tract⁹; synthesis of prostaglandin E₂, which is involved in cervical ripening; myometrial contraction during labor¹⁰; and induction of differentiated trophoblast functions, such as production and secretion of human chorionic gonadotropin and human placental lactogen.^{11, 12}

These biologic actions of EGF are thought to be mediated by its binding to EGF receptors that are present in the target cells. The EGF receptor is known to be a glycoprotein of 170,000 d molecular weight and is present in a variety of cell types. We have immunohistochemically documented that the EGF receptor in 6- to 40-week human placentas is almost exclusively localized to syncytiotrophoblasts and that immunohistochemically detected cellular levels of EGF receptor in the placenta vary considerably over the course of pregnancy.¹³

Although placental materials are most available between 6 and 12 weeks' gestation and at term, we attempted to obtain placental tissue samples representing the entire course of pregnancy. In this study attention was focused on the expresson of EGF and EGF receptor in very early placenta before 6 weeks' gestation and the changes in cytologic localization of EGF and EGF receptor observed over the course of pregnancy. This has

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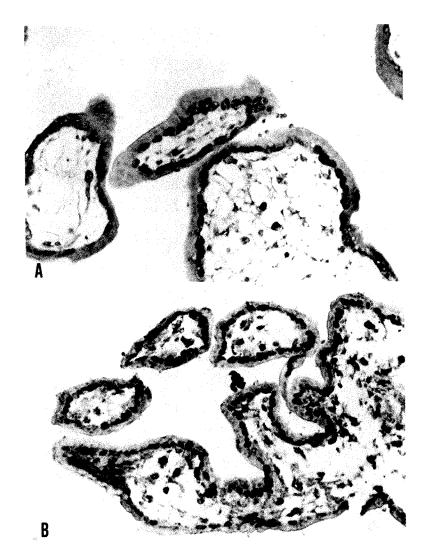


Fig. 1. Immunohistochemical localization of EGF and EGF receptor in very early first-trimester placenta. Formalin-fixed paraffin-embedded sections of 4-week placenta were stained with anti-EGF antibody (A) and with anti-EGF-receptor antibody (B). Cytotrophoblastic cells showed pronounced staining with anti-EGF antibody and with anti-EGF-receptor antibody, whereas syncytiotrophoblasts were negative for appreciable staining. (Original magnification $\times 200$.)

been discussed in relation to the possible involvement of the EGF system in fetoplacental development.

Material and methods

Materials. First-trimester placentas were obtained from 18 patients who underwent elective abortion at 4 to 5 weeks (three cases), 6 to 7 weeks (five cases), 8 to 9 weeks (six cases), and 10 to 12 weeks (four cases) of gestation. Second-trimester placentas were obtained from three patients who underwent elective abortion between 16 and 21 weeks, whereas third-trimester placentas were obtained from four patients who had cesarean section between 38 and 40 weeks. The gestational age of the placenta was determined by the known date of conception or by estimation from the date of

the patient's last menstrual period. In all cases gestational age was further evaluated by ultrasonographic examination. Informed consent was obtained before operation from the patients for the use of placental tissues for immunhistochemical studies.

Immunohistochemical staining. Placental tissues were fixed in 4% buffered neutral formalin, dehydrated, and embedded in paraffin. Sections, 5 to 6 μ m in thickness, were deparaffinized and followed by standard histologic techniques.

Immunohistochemical staining was performed by avidin-biotin immunoperoxidase techniques with the use of a polyvalent immunoperoxidase kit (Omnitags, Lipshaw, Mich.) as previously described by Maruo and Mochizuki. A rabbit polyclonal antibody against EGF



Fig. 2. Immunohistochemical localization of EGF and EGF receptor in early first-trimester placenta. Formalin-fixed, paraffin-embedded sections of 7-week placenta were stained with anti-EGF antibody (A) and anti-EGF-receptor antibody (B). Pronounced staining for EGF and EGF receptor was observed in syncytiotrophoblasts, whereas cytotrophoblastic cells were negative for appreciable staining. (Original magnification $\times 200$.)

(Collaborated Research Inc., Bedford, Mass.) and a mouse monoclonal antibody against human EGF receptor (Transformation Research Inc., Framingham, Mass.) were used as the primary antibodies in this study. The anti-EGF antibody and anti-EGF-receptor antibody were diluted 1:50 and 1:10 before use, respectively.

To assure specificity of the immunologic reactions, adjacent control sections were subjected to the same immunoperoxidase method, with the exception that the primary antibody to EGF or EGF receptor was respectively replaced by nonimmune rabbit immunoglobin G (Miles, Elkhart, Ind.) or nonimmune murine immunoglobin G (Miles). In the controls no positive staining was observed.

Results

Fig. 1, A and B, demonstrates the immunohistochemical staining for EGF and EGF receptor, respectively, in very early first-trimester placentas obtained at 4 weeks of gestation. Immunostaining with both anti-EGF antibody and anti-EGF-receptor antibody was almost exclusively localized to cytotrophoblasts. Neither syncytiotrophoblasts nor interstitial cells of placental villi showed any appreciable staining for EGF and EGF receptor. A similar pattern of cytologic localization of EGF and EGF receptor was observed in tissue sections of 5-week placentas.

Fig. 2, A and B, shows the immunohistochemical staining for EGF and EGF receptor, respectively, in early first-trimester placentas obtained at 7 weeks of

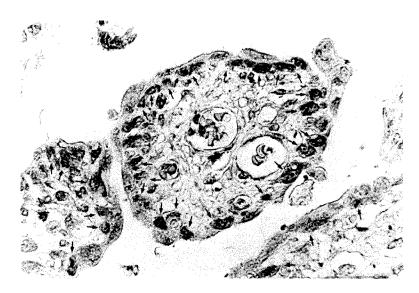


Fig. 3. Immunohistochemical localization of EGF in second-trimester placenta. Formalin-fixed, paraffin-embedded sections of 21-week placenta were stained with anti-EGF antibody. Prominent staining for EGF was observed in cytotrophoblastic cells. (Original magnification ×400.)

Table I. Immunocytologic reaction for EGF and EGF receptor in developing human placenta

			Immunostain	ing intensity	
Castational	No Commissed		EGF	EGF	receptor
Gestational age (wk)	No. of examined cases	Cytotrophoblast	Syncytiotrophoblast	Cytotrophoblast	Syncytiotrophoblas
4-5	3	+++	, march	+++	
6-7	5		+++) Profes	+++
8-9	6	****	+++	www.	+++
10-12	4	Norw	++		++
16-21	3	+	quinte	****	+
38-40	4	+	Market	- Seener	+

gestation. Unlike the very early first-trimester placentas obtained at 4 to 5 weeks of gestation, immunostaining with both anti-EGF antibody and anti-EGF-receptor antibody was predominantly localized to syncytiotrophoblasts. A similar pattern of cytologic localization of EGF and EGF receptor was found in tissue sections of 8- to 12-week placentas.

In the case of second-trimester placentas obtained between 16 and 21 weeks of gestation, prominent staining with anti-EGF antibody was observed in cytotrophoblasts (Fig. 3), whereas immunostaining with anti-EGF-receptor antibody has been predominantly noted in syncytiotrophoblasts.

Fig. 4 represents the immunohistochemical staining for EGF in third-trimester placentas obtained at 40 weeks of gestation. Immunostaining with anti-EGF antibody was pronounced in cytotrophoblasts, whereas immunostaining with anti-EGF-receptor antibody has been found in syncytiotrophoblasts.

The intensity of immunostaining of placental trophoblasts for EGF and EGF receptor varied over the course of pregnancy. The most pronounced staining was obtained in cytotrophoblasts of very early first-trimester (4- to 5-week) placentas and in syncytiotrophoblasts of early first-trimester (6- to 8-week) placentas. The staining intensity in second-trimester placental trophoblasts declined compared with that in first-trimester placentas, and third-trimester placental trophoblasts displayed even less staining for EGF and EGF receptor (Table I).

Comment

The data presented in this paper demonstrate that cytologic localization of EGF and EGF receptor in developing human placentas varies over the course of pregnancy. Even during the first trimester, a remarkable change in cytologic localization of EGF and EGF receptor in the placenta appeared between 5 and 6 weeks of gestation. In the very early placenta obtained at 4 to 5 weeks, EGF and EGF receptor were almost exclusively localized to cytotrophoblasts, whereas EGF and EGF receptor in early placentas obtained between

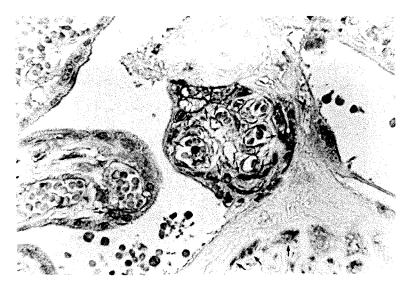


Fig. 4. Immunohistochemical localization of EGF in third-trimester placenta. Formalin-fixed, paraffin-embedded sections of 40-week placenta were stained with anti-EGF antibody. Prominent staining for EGF was found in cytotrophoblastic cells. (Original magnification ×400.)

6 and 12 weeks were predominantly localized to syncytiotrophoblasts. These findings suggest that both EGF and EGF receptor are initially expressed in cytotrophoblasts in very early placentas before 6 weeks of gestation and thereafter expressed in syncytiotrophoblasts of 6- to 12-week placentas. To our knowledge, this is the first report to demonstrate the simultaneous expression of EGF and EGF receptor in cytotrophoblasts as early as 4 to 5 weeks of gestation. The change of cytologic localization of EGF and EGF receptor in first-trimester placentas is of great interest, considering the established finding that syncytiotrophoblast is formed from cytotrophoblast by a process of proliferation followed by cell fusion.14.15

The fact that both EGF and EGF receptor in 4- to 5week placentas were localized to mitotically active cytotrophoblasts suggest that, in very early placentas before 6 weeks of gestation, EGF and EGF receptor may be linked to the proliferation of cytotrophoblasts. Although the mechanisms by which the mitogenic effects of EGF on very early placental cytotrophoblasts are mediated are unclear, it is conceivable that the mitogenic effects may be mediated by the expression of a protooncogene, c-mye. We have recently demonstrated that immunoreactive c-myc protein product appears in the nuclei of cytotrophoblasts. Furthermore, as cytotrophoblasts are in close contact with the embryonic disk,16 the predominance of cytologic localization of EGF and EGF receptor in cytotrophoblasts of very early placentas is likely to be consistent with the demands of early embryonic development as well.

On the other hand, the fact that both EGF and EGF receptor in 6- to 12-week placentas were localized to mitotically inactive syncytiotrophoblasts suggests that

EGF and EGF receptor in early placentas between 6 and 12 weeks of gestation may be involved in the induction of differentiated trophoblast function. In accordance with this, EGF has been demonstrated to stimulate human chorionic gonadotropin production by cultured early placental tissues obtained at 7 to 8 weeks of gestation.¹² Thus the predominance of cytologic localization of EGF and EGF receptor in syncytiotrophoblasts of 6- to 12-week placentas seems to relate to the formation of the peak of increased human chorionic gonadotropin production and secretion by early placenta.

The placenta is known to take over the corpus luteum function in producing progesterone and estrogen at the gestational age of 6 weeks. Before the sixth week of gestation, the corpus luteum is the prime site of progesterone and estrogen production,17 and after the sixth week of gestation the placenta becomes the main site of progesterone and estrogen production. The dynamic change of cytologic localization of EGF and EGF receptor in the placenta observed at the gestational age of 6 weeks is consistent with placental steroid hormone production at 6 weeks. This change of cytologic localization of EGF and EGF receptor from cytotrophoblasts to syncytiotrophoblasts observed in early pregnancy may reflect a possible change of the role of EGF and EGF receptor in the process of fetoplacental development. It is likely that EGF in the placenta before 6 weeks' gestation may play a major role in the induction of trophoblast proliferation and early embryonic development rather than placental hormone production, whereas EGF in the placenta between 6 and 12 weeks' gestation may play a major role in the induction of placental hormone production.

The simultaneous expression of EGF and EGF receptor in the cytotrophoblast of 4- to 5-week placentas and in the syncytiotrophoblast of 6- to 12-week placentas raises a possibility that EGF in first-trimester placentas may act in an autocrine manner. By contrast, in second-trimester and third-trimester placentas, EGF was predominantly localized to cytotrophoblasts, whereas EGF receptor was localized to syncytiotrophoblasts. This suggests that EGF in second- and thirdtrimester placentas may act in a paracrine fashion. Although the possible role of EGF and EGF receptor in second- and third-trimester placentas is still unknown, it is tempting to speculate that EGF and EGF receptor in second- and third-trimester placentas may be linked to the induction of cellular differentiation and human placental lactogen secretion, as suggested by Morrish et al.18 and Maruo et al.12 Further studies will be needed to elucidate the vital roles of EGF and EGF receptor in the fetoplacental development throughout pregnancy.

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Case report of myocardial infarction in labor

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Substernal and epigastric pain occurring during labor was not considered important until the first postpartum day when an obvious myocardial infarction was diagnosed in a 37-year-old woman. Angiography revealed normal coronary arteries, and the myocardial infarction was postulated to be due to arterial spasm in association with smoking, oxytocin, ephedrine, and epidural anesthesia. (AM J OBSTET GYNECOL 1991;165:1383-4.)

Key words: Pregnancy, infarction, oxytocin, arterial spasm

Myocardial infarction during pregnancy and the puerperium is rare; its diagnosis is often overlooked because of its rarity and vague clinical presentation.

Case report

A 37-year-old white primigravid woman at 39 weeks' gestation was seen in early labor. The patient's medical history was unremarkable except for smoking one pack of cigarettes per day. She had no history of hypertension, cardiac disease, or hypercholesterolemia. She had no history of cocaine use. There were no prenatal complications when she was seen over a period of nine visits.

On admission, she was in good general health with ruptured membranes and mild uterine contractions every 5 minutes. Blood pressure was 130/80 mm Hg and pulse was 100 beats/min and regular. A grade 1/6 systolic ejection murmur was heard at the upper left sternal border. All routine laboratory data were normal. Intravenous oxytocin (Pitocin) infusion was started 8 hours after admission and reached a maximum dose of 8 mU/min. She experienced "heartburn" 21/2 hours later. This was thought to be secondary to gastroesophageal reflux and resolved with a suspension of aluminum and magnesium hydroxides (Maalox) and emesis. She received a total of 870 mU of oxytocin over this time. The infusion was stopped for an epidural anesthetic to be administered (15 ml of 0.25% bupivacaine hydrochloride [Marcaine]). Blood pressure dropped to 80/50 mm Hg with a pulse of 80 beats/min 10 minutes after the anesthetic was given; this was followed by further episodes of emesis. She was given 25 mg promethazine hydrochloride and 20 mg of ephedrine sulfate intravenously, and blood pressure returned to 100/60 mm Hg. Intravenous oxytocin was restarted, and there were no further episodes of chest pain. At

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12:30 AM she was spontaneously delivered of a 7 pound 5 ounce male infant with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively.

At 1:30 AM, 2 days post partum, she complained of severe "heartburn" radiating to the back and left arm and "a large weight on her chest." She denied shortness of breath and dizziness but was diaphoretic. On physical examination, blood pressure was 150/90 mm Hg, pulse 80 beats/min and regular, respirations 16 breaths/min, and a 2/6 systolic ejection murmur at the upper left sternal border, but no third or fourth heart sounds were heard. She was treated with aluminum hydroxide and magnesium trisilicate (Mylanta) and acetaminophen and codeine phosphate (Tylenol #3). The pain, which resolved after I hour, was believed initially to have been esophagitis or musculoskeletal pain. However, an electrocardiogram done at 10:00 AM revealed an anterolateral myocardial infarction with Q waves in leads III and V2-V5, inverted T waves in lead AVL, and significant ST elevations in leads V2-V5. Echocardiogram demonstrated hypokinesis of the left ventricle. The cardiac enzyme pattern confirmed the diagnosis and suggested that the infarction had occurred 2 to 3 days previously (presumably during labor). The second episode of chest pain was presumed to be postinfarction angina. Coronary angiography was done 5 days after infarction; normal coronary arteries were demonstrated. Blood cholesterol was 250 mg/dl. The patient was treated with rest, anticoagulant therapy, warfarin sodium (Coumadin), and calcium-channel blockers (diltiazem hydrochloride). She was discharged on the eighth hospital day.

Comment

A recent review by Hankins et al. found 70 cases of myocardial infarction in pregnancy reported in the world literature. Mortality attributable to myocardial infarction in pregnancy and the puerperium is estimated to be 30% to 37%. Unfortunately, because of the rarity of the event and the age and sex of the patient, the symptoms of an impending infarction are often mistaken for more common and benign conditions typically associated with pregnancy.

The most common risk factor in young patients with

myocardial infarction is cigarette smoking. The risk of myocardial infarction in women <50 years old increases with the number of cigarettes smoked per day. Hypertension was present in 25% of patients with myocardial infarction occurring during pregnancy and the puerperium. Among patients with myocardial infarction, normal coronary angiograms are estimated at 17% for patients under the age of 36.

A likely explanation for myocardial infarction with normal coronary arteries is coronary artery spasm, the exact cause of which is unknown. The hemodynamic changes that occur in late pregnancy when the patient is in the supine position cause chorionic ischemia and may result in uterine renin release and angiotensin production with subsequent coronary artery spasm. Direct vasospastic effect of cigarette smoking on the coronary arteries has been demonstrated during angiography. The synthetic oxytocin used for this patient is devoid of vasopressin yet still has inherent pressor properties.

Evron et al.² have reported a case in which severe local arteriospasm followed infusion of the routine minimal dose of oxytocin required for postpartum uterine contraction. Therefore it is conceivable that oxytocin, given in high doses, also could cause coronary artery spasm.

The cause of this patient's myocardial infarction, in the presence of normal coronary arteries, remains unclear. Possible explanations include supine hypotension, epidural anesthesia, an injection of ephedrine, cigarette smoking, and intravenous oxytocin, each of which, individually or in combination, may have been responsible.

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Endocrine effects in female weight lifters who self-administer testosterone and anabolic steroids

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It appears that the self-administration of testosterone and anabolic steroids is increasingly practiced by women in sports where strength and endurance are important. We recently evaluated endocrine parameters in nine female weight lifters using steroids and seven not using these agents. Of the nine anabolic steroid users, seven took multiple anabolic steroids simultaneously. Thirty-fold elevations of serum testosterone were noted in the women injecting testosterone. In three of these women serum testosterone levels exceeded the upper limits for normal male testosterone concentrations. A significant compensatory decrease in sex hormone—binding globulin and a decrease in thyroid-binding proteins were noted in the women steroid users. Also, a 39% decrease in high-density lipoprotein cholesterol was noted in the steroid-using weight lifters. Most of the subjects in this study used anabolic steroids continuously, which raises concern about premature atherosclerosis and other disease processes developing in these women. (AM J OBSTET GYNECOL 1991;165:1385-90.)

Key words: Weight lifers, women, testosterone, anabolic steroids, endocrine effects

The adverse effects of anabolic steroids are well described in men. These include endocrinologic (decrease in plasma testosterone and gonadotropin levels, decrease or absence of spermatogenesis, testicular atrophy, and gynecomastia), metabolic (decreased high-density lipoprotein [HDL] cholesterol, increased low-density lipoprotein [LDL] cholesterol, and hypertension), hepatic (hepatitis, cholestasis, benign and malignant tumors), and adverse psychologic effects. 1-3

The effects of androgens vary significantly among users and reflect different influences of the particular androgen preparation and individual differences in response. ^{4,5} The long-term risks of androgen use are unknown. ⁶ Also, many of these studies do not evaluate androgenic steroids as they are commonly taken, where several types of oral and injectable androgens are taken simultaneously (called stacking). This practice may produce androgen levels 10 to 100 times the replacement dose. ¹

There have been few studies in women steroid users, but the side effects that have been reported include hirsutism, deepening of the voice, hypertrophy of the clitoris, male pattern baldness, acne, menstrual abnormalities, increased aggressiveness, and, most recently, changes in lipoprotein profiles.^{7,8}

We recently had the opportunity to examine endocrinologic parameters in female weight lifters who were self-administering multiple androgenic steroids in preparation for competition.

Material and methods

Subjects. The study population consisted of nine female weight lifters using androgens and seven female weight lifters in the follicular phase of the menstrual cycle who were not using androgens (Table I). They were evaluated at The Ohio State University Clinical Research Center after informed consent and assurances of confidentiality were obtained. A patient interview, a detailed questionnaire, and a blood specimen were acquired. These women had no history of illness in the past month, other than upper respiratory infections. They were nonsmokers and alcohol intake was modest. The most frequently used steroids were methandrostenolone (Dianabol), stanozolol (Winstrol), nandrolone decanoate (Deca-Durabolin), oxandrolone (Anavar), and injectable testosterone preparations. Also used were mibolerone, methenolone acetate, trenbolone acetate, and Parabolin. These athletes obtained their steroids by nonprescription methods. A blood sample was obtained when androgen use was at its peak and also when androgen use ceased. However, only three subjects stopped steroids completely in a 12month period, for 3, 4, and 9 weeks, respectively.

Assays. Laboratory studies included a complete blood cell count and chemical analyzer measurements

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Table I. Characteristics of weight lifters in relation to androgen use

					D		Androgens	
Subject No.	Age (yr)	Weight (kg)	Height (cm)	Training (yr)	Drug use (yr)	Menses	Oral	Injectable
1	29	62.7	162.6	12	7.0	Oligomenorrhea	2	2
2	28	58.6	157.5	7	5.0	Oligomenorrhea	1	3
3	41	77.3	167.6	7	5:0	Amenorrhea	1	3
4	40	68.2	170.2	5	4.0	Oligomenorrhea	1	1
5	30	68.2	160.0	5	4.5	Amenorrhea	2	1
6	35	51.8	152.4	7	5.0	Normal	2	1
7	26	52.3	160.0	4	3.5	Normal	1	0
8	23	75.5	162.6	3	0.25	Oligomenorrhea	. 2	2
9	33	60.9	160.0	5	3.0	Oligomenorrhea	0	1
Mean ± SE	$31.7 \pm 2*$	63.9 ± 3.1	$161.4 \pm 1.8*$	$6 \pm 1*$	4 ± 0.6	tenders.	1.3 ± 0.2	1.6 ± 0.3
Nonusers	25 ± 2	60 ± 6.2	168 ± 2.0	3 ± 0.7	NA	Normal	NA	NA

NA, Not applicable.

Table II. Effect of self-administered androgens on gonadtropins and sex steroid metabolism in women

Subject No.	Follicle- stimulating hormone (IU/L)	Luteinizing hormone (IU/L)	Estradiol (pmol/L)	Dehydro- epiandrosterone sulfate (nmol/L)	SHBG (nmol/L)	Testosterone (nmol/L)
1	12	14	242.3	3.2	. 14.4	39.2
2	· 3	3	290.0	3.6	24.2	66.2
· 3	3 ·	. 3	146.8	2.1	10.3	75.9
4	4	3	290.0	3.0	27.3	139.4
5	6	. 3	36.7	1.7	21.4	44.4
6	12	3	157.9	9.2	27.0	35.4
7.	14	11	205.6	2.7	47.3	1.4
8	3	3	216.6	5.7	9.3	27.7
9	10	4	132.2	1.4	20.2	1.9
Mean ± SE	$7.4 \pm 1.5*$	5.2 ± 1.4	190.9 ± 27.2	3.6 ± 0.81	$22.4 \pm 3.8*$	$47.9 \pm 14.2*$
Nonusers	12.3 ± 1.1	6.7 ± 1.1	133 ± 26.1	4.2 ± 0.4	52.5 ± 6.4	1.2 ± 0.1
Normal range	<17	<25	110-367.1	2.7-9.5	20-120	0.69-2.77

^{*}Significantly different from controls (p < 0.05).

of serum glucose, creatinine, blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and creatine kinase. Also a lipid profile (HDL, total cholesterol, and triglyceride) was obtained with a Kodak Ectachem with barium sulfate precipitation before the HDL determination. The hormonal evaluation consisted of radioimmunoassays with standardized measurements for luteinizing hormone, follicle-stimulating hormone, estradiol, prolactin, growth hormone, dehydroepiandrosterone sulfate, sex hormone-binding globulin (SHBG), testosterone, thyroxine, and thyroid-stimulating hormone, as performed in our endocrine laboratories.9-12 Thyroxinebinding proteins were measured by fluorescence polarization (T-Uptake, Abbott Laboratories, Chicago), which provides an index as an approximation of thyroxine binding in serum. The intraassay and interassay coefficients of variation for these assays were <12%. The data were evaluated by nonpaired t tests; p < 0.05

was considered significant. Urine was not assayed for the presence of anabolic steroids.

Results

Subject characteristics. The nine weight lifters using steroids were between 23 and 41 years old (Fable I) and had been weight training 3 to 12 years. They had been using androgens for 3 months to 7 years, and all but two of the women had menstrual abnormalities (Table I). The weight-training women who were not steroid users had been weight lifting for I to 6 years and all had normal menses (Table I). The women who stated they were using three or more steroid preparations believed they had increased strength, muscle size, enhancement of training intensity, and improved performance while taking these agents. They also noted an increase in appetite, aggressiveness, irritability, sex drive, acne, deepening of the voice, body hair, and clitoral size. The serum chemistry profile demonstrated

^{*}Significantly different from controls (p < 0.05).

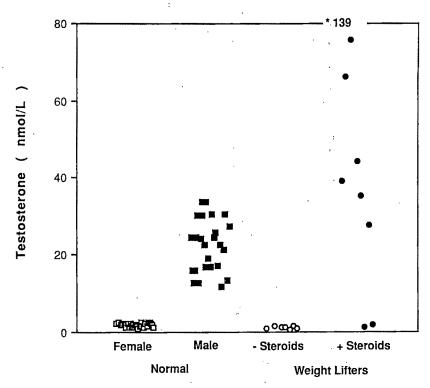


Fig. 1. Serum testosterone levels were elevated in seven female weight lifters who were injecting testosterone and were normal in two women who were using only other anabolic steroids. Note that several testosterone levels in women users were higher than those found in normal men.

mild elevations of levels of creatine kinase (in eight steroid users and two nonusers), alanine aminotransferase or aspartate aminotransferase (in four steroid users), and serum creatinine (in four steroid users) (data not shown). Other serum chemistry values were normal, including the γ -glutamyltranspeptidase and alkaline phosphatase levels.

Testosterone and SHBG levels. The most dramatic finding in the steroid users was the marked elevation in serum testosterone levels noted in seven of the nine women (Table II). The mean serum testosterone levels were 30 times those found in the non-steroid-using, weight-lifting women or in the normal female population (Fig. 1). Three of the women steroid users (subjects 2 to 4, Table II) had serum testosterone levels above the upper limits of normal for men (Fig. 1). The two women steroid users with normal serum testosterone levels were not injecting themselves with testosterone (subjects 7 and 9, Table II).

An appropriate decrease in SHBG was produced by testosterone and the other anabolic steroids (Table II). The SHBG levels (mean \pm SE) in the women weight lifters who used steroids (22.4 \pm 3.8) were significantly less than those found in normal women (57.0 \pm 4.1, p < 0.0001), women weight lifters who were not androgen users (52.5 \pm 6.4, p < 0.02), and normal men (59.6 \pm 6.5, p < 0.002) (Fig. 2). The women using the greatest number of androgen preparations (subjects 1,

3, and 8, Table I) had the lowest SHBG levels (Table II). Three women users (subjects 1-3, Table I) stopped androgens for 3 to 9 weeks. At 3 weeks the testosterone level was still elevated at 14.2 nmol/L in subject 1. At 4 and 9 weeks, however, the other subjects had normal serum testosterone levels. These latter women in whom serum testosterone levels normalized continued to have suppressed SHBG levels of 6 and 29 nmol/L.

Other endocrine measures. No significant differences (mean ± SE) in luteinizing hormone and dehydroepiandrosterone sulfate levels were found between the weight lifters who were users and nonusers of androgens, but follicle-stimulating hormone levels were significantly (p < 0.03) lower in the steroid-using women (Table II). Prolactin (users vs nonusers, 5.3 ± 0.1 and $8.0 \pm 1.0 \mu g/L$) and growth hormone (users vs nonusers, 7.4 ± 3.0 and $6.6 \pm 3.7 \mu g/L$) levels (mean \pm SE) were not significantly influenced by steroid use. The values (mean \pm SE) of total cholesterol (users vs nonusers, 4.4 ± 0.4 and 4.0 ± 0.2 nmol/L), LDL cholesterol (users vs nonusers, 3.3 ± 0.4 and 2.4 ± 0.1 nmol/L), and triglyceride (users vs nonusers, 0.8 ± 0.1 and 1.0 ± 0.2 nmol/L) were not different (Table III). The HDL cholesterol levels (mean \pm SE), however, were significantly (p < 0.05) depressed in the androgen users (users vs nonusers, 0.9 ± 0.1 and $1.5 \pm 0.1 \text{ nmol/L}$) (Table III).

Serum thyroxine and thyroxine-binding protein

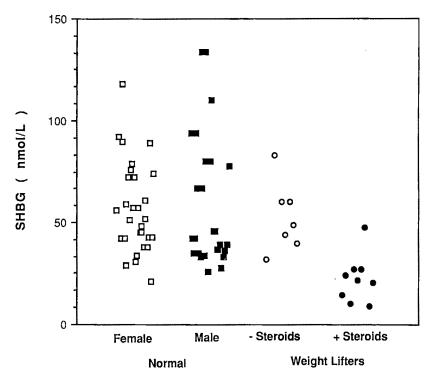


Fig. 2. SHBG levels were significantly (p < 0.02) decreased in women who were using anabolic steroids.

Table III. Effect of self-administration of androgens on serum lipid levels in women

Subject no.	Cholesterol (mmol/L)	HDL (mmol/L)	$LDL \ (mmol/L)$	Triglycerides (mmol/L)
1	5.69	1.01	4.52	0.82
2	4.47	1.22	3.13	0.60
3	. 6.23	0.52	5.48	1.13
4	3.49	0.75	2.66	0.42
. 5	3.80	0.70	2.94	0.82
6	3.98	1.47	2.45	0.30
7	4.06	0.91	2.98	- 0.87
8	2.43	0.67	1.67	0.43
9	5.40	1.06	4.08	1.32
Mean ± SE	4.4 ± 0.4	$0.9 \pm 0.1*$	3.3 ± 0.4	0.8 ± 0.1
Nonusers ± SEM	4.0 ± 0.2	1.5 ± 0.1	2.4 ± 0.1	1.0 ± 0.2
Normal range	3.31-5.40	1.24-2.25	1.71-3.59	0.56-2.26

^{*}Significantly different from controls (p < 0.05).

levels were significantly (p < 0.05) decreased in the androgen-using women (Table IV). The thyroid-stimulating hormone level (mean \pm SE) was significantly (p < 0.001) higher in the androgen-using women (Table IV).

Comment

The major finding in this study is the marked elevation in serum testosterone levels in the female weight lifters who were injecting this sex steroid. Several of the testosterone levels obtained were higher than those usually found in normal male control subjects (Fig. 1). Their serum testosterone levels on any given day would be a reflection of the dose injected and the length of the interval between injection and blood sampling.

The biologic significance of these high levels and the other androgens not measured was documented by several lines of evidence. First, the androgen users had features of hyperandrogenism including hirsutism, increased muscular development, acne, clitoromegaly, and menstrual disturbances. Second, they had bio-

Subject No.	Thyroxine (nmol/L)	Thyroxine-binding index	Free thyroxine concentration	Thyroid- stimulating hormone (mU/L)
1	78.5	0.80	7.6	4.1
2	75.9	0.75	7.9	2.6
3	32.2	0.32	7.8	3.9
4	19.1	0.71	8.7	2.1
5	60.5	0.40	11.8	1.4
6	64.4	0.66	7.6	1.4
7	73.4	0.80	7.1	3.0
8	43.8	0.39	8.7	1.3
9	55.3	0.65	6.6	2.9
Mean ± SE	$56 \pm 7*$	$0.6 \pm 1*$	$8.2~\pm~0.5$	$2.5~\pm~0.4*$
Nonusers	85 ± 5	1 ± 0.02	6.3 ± 0.4	0.8 ± 0.1
Normal range	58-161	0.7-1.25	3.5-11.0	0.4-4.0

Table IV. Influence of self-administration of androgens on thyroid function tests in women

chemical changes that included a decrease in SHBG and HDL cholesterol levels.

SHBG is a protein that is produced in the liver and binds 60% of the circulating testosterone. The concentration of SHBG has been shown to be lowered by testosterone14 and other anabolic steroids15 by means of their actions on the liver. We saw similar effects in our subjects. A recent report of men taking various anabolic steroids13 and women using stanozolol and danazol14, 15 also showed a decrease in SHBG levels. Additionally, the diminished estrogen effect in seven of nine steroid users who had oligomenorrhea or amenorrhea probably contributed to the decreased SHBG levels.

Serum estradiol concentrations in the women steroid users approximated the midfollicular levels of the control women, which would be expected in oligomenorrheic and amenorrheic women. The mean follicle-stimulating hormone level of the androgen using women was suppressed (Table II), and their luteinizing hormone levels were similar to those of the controls. It is also possible that in the steroid users testosterone and the other anabolic steroids were interfering with the amplitude or number of gonadotropin-releasing hormone pulses thus producing anovulatory cycles.

Thyroxine-binding proteins also were decreased in the steroid users, as reflected by the low thyroxinebinding index and the decrease in total serum thyroxine levels (Table III). These latter changes had no significant influence on the biologic activity of thyroid hormone, however, because the free thyroxine concentration and the thyroid-stimulating hormone level were within normal limits. These findings are similar to those of a previous report of decreased thyroxine-binding globulin in men who were using anabolic steroids.13

The marked (39%) decrease in serum HDL levels in women androgen users in our study is similar to that previously reported in women and men who used steroids to enhance performance.1,8 The diminished estrogen activity that is associated with the oligomenorrhea and amenorrhea that most of the steroid users experienced probably contributed to the decreased HDL cholesterol level.

Also, the fall in HDL produced by anabolic steroids may be related to a coincidental increase in hepatic triglyceride lipase that may remove circulating HDL particles.16 The suppression of HDL cholesterol is less after testosterone injection than after the anabolic steroid regimens. It appears that the explanation for this latter observation is that the lipoprotein alterations are peculiar to the anabolic steroids with a 17α-alkylated structure.17, 18

We observed an increase in LDL cholesterol levels in the steroid users that was similar to that of previous reports,1,8 but the increase was not statistically significant (p < 0.06).

Several of the women steroid users in this study had an elevation of alanine aminotransferase, aspartate aminotransferase, and creatine kinase levels, but the alkaline phosphatase and y-glutamyltranspeptidase levels were normal. In four of these patients with the highest elevations of transaminase we also noted an increase in serum creatinine levels. Thus the evidence would suggest muscle rather than liver as the primary source of the elevation in enzymes. An increase in serum transaminase levels previously has been reported by our group in weight lifters who were not using anabolic steroids.19 This latter increase was not seen in the non-steroid-using weight lifters in this study, perhaps reflecting a less intense lifting regimen.

In conclusion, most of the subjects in this study were continuously self-administering multiple androgens, which raises concern that the abnormalities found in these women could predispose them to premature atherosclerosis and other disease processes.

^{*}Significantly different from controls (p < 0.05).

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The effect of gestational age on the detection rate of Down's syndrome by maternal serum α -fetoprotein screening

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Low levels of maternal serum α -fetoprotein are currently being used to screen for Down's syndrome in midpregnancy. Because of the possibility that gestational age may affect the detection rate of Down's syndrome, we analyzed maternal serum AFP levels and gestational age in 51 Down's syndrome pregnancies that had been confirmed by amniocentesis or at birth, and we compared these pregnancies with 3239 screened singleton pregnancies with known normal outcomes. The highest yield of a low risk for Down's syndrome associated with maternal serum α -fetoprotein occurred at 16.5 to 17.5 weeks' gestation. Our data suggest that maternal serum α -fetoprotein screening for Down's syndrome should be done between 16 and 18 weeks' gestation, which is the gestational age currently recommended for neural tube defect screening. (AM J OBSTET GYNECOL 1991;165:1391-3.)

Key words: α-Fetoprotein, Down's syndrome, gestational age, prenatal screening

Since the initial observation of low levels of maternal serum α-fetoprotein (AFP) that occurred in Down's syndrome pregnancies in 1984,1 low levels of maternal serum AFP in conjunction with maternal age2 have been increasingly used to screen for Down's syndrome in midpregnancy.3 Several reports have recommended that maternal serum α-fetoprotein evaluation should not be repeated after an initial low level is identified.4 This is based on observations of normal maternal serum AFP levels in second samples from Down's syndrome pregnancies after an initial low level, which is a phenomenon thought to result from regression to the mean. Because of the possibility that gestational age also may affect the detection rate of Down's syndrome, we analyzed data from the Baylor Maternal Serum Alpha-Fetoprotein Screening Program to study this question. In an earlier preliminary study we found a decrease in Down's syndrome detection rate with increasing gestational age.5

Material and methods

The Baylor Maternal Serum Alpha-Fetoprotein Screening Program is a nonprofit medical school based prenatal screening program that has been in op-

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eration since 1982. Women screened by the program come mainly from the Houston area, as well as regionally. Low maternal serum AFP levels have been used to screen for increased risk of fetal Down's syndrome since 1985, and >45,000 women have been screened.

AFP was measured by radioimmunoassay with reagents from Clinical Assays, Cambridge, Mass. A more sensitive radioimmunoassay was obtained by changing the conditions of the assay to derive a standard curve from 1.0 to 50 ng/ml. The intraassay and interassay coefficient of variation at this low level was <5%. The AFP concentrations were converted to multiples of the normal median as a result of our own previous data and were adjusted for maternal weight by means of previously published formulas.⁶ A risk of Down's syndrome was then calculated by computer with the a priori maternal age risk.⁷ A cutoff risk of 1:360 at delivery was used. Gestational age was calculated by the last menstrual period unless the gestational age calculated by biparietal diameter was discrepant by >1 week.

We ascertained 52 screened Down's syndrome pregnancies since 1985. Of these 52 pregnancies, 51 were singleton gestations. Twenty-five of those women had low maternal serum AFP levels (Down's syndrome risk >1:360); they were offered amniocentesis, which confirmed prenatally that each patient had a fetus with Down's syndrome. The remaining 26 pregnancies had "normal" maternal serum AFP levels but resulted in a Down's syndrome infant at delivery. This may represent an underascertainment of Down's syndrome pregnancies that are undetected by maternal serum AFP screening; 50 to 60 such pregnancies are expected.8

	Maternal serum AFP (multiples of the median)					
Don't a set sout at in	Case	· ·	Contr	ols		
Duration of gestation (wk)	Mean ± SD	Range	Mean ± SD	Range		
14-16.5	0.74 + 0.34 $(n = 1)$	0.24-1.81	1.01 + 0.57 . $(n = 13)$	0.21-5.35		
15.5-17.5	$0.59 + 0.22' \qquad (n = 1)$	0.27-1.04	0.98 + 0.54 = 0.98 + 0.54	0.14-5.57		
17.5-21.5	0.69 + 0.33 (n =	0.28-1.38	1.04 + 0.53 $(n = 8)$	0.22-5.71		

Table I. Maternal serum AFP values (in multiples of the median) for Down's syndrome pregnancies compared with controls

Table II. Results stratified by gestational age intervals and corresponding tests for association

	Muli of med	tĥe	
	<0.8	>0.8	Totals
14.0-<16.5 Weeks' ge	estation		
No. of cases	16	6	2
No. of controls	605	729	1334
Odds ratio	3.3	21	
95% Confidence interval	1.25	-8.26	
16.5-<17.5 Weeks' ge	estation		
No. of cases	12	2	14
No. of controls	488	590	1078
Odds ratio	7.3	25	
95% Confidence interval	1.62	-32.57	
17.5-21.5 Weeks' gest	tation		
No. of cases	9	6	15
No. of controls	322	497	819
. Odds ratio	2.	32	
95% Confidence interval	0.82	-6.57	

The 51 singleton Down's syndrome pregnancies studied constituted the case group. The control group consisted of 3239 randomly selected previously screened women with documented known normal pregnancy outcomes. Data from both groups were abstracted from the Baylor Maternal Serum Alpha-Fetoprotein Screening Program computer files.

To examine the effect of maternal serum AFP on Down's syndrome risk, cutoff points of <0.8 and ≥0.8 multiples of the median were selected to maximize specificity and sensitivity. To evaluate the modifying effect of gestational age on the association between maternal serum AFP and Down's syndrome, we stratified the maternal serum AFP data by gestational age in three categories: 14 to <16.5 weeks, 16.5 to <17.5 weeks, and 17.5 to 21.5 weeks. Although we originally stratified the data into narrower time windows, we found

that the effects were highly consistent within the three larger strata, which made it possible to gain precision (by collapsing the strata) with no loss in validity. We computed standard odds ratios and 95% confidence intervals (logit transformation) and applied the Breslow-Day test for homogeneity across strata (SAS version 6.04 PROC FREQ; SAS Institute, Inc., Carey, N.C.).

The data also were entered into a multiple logistic regression model (SAS version 6.04 PROC LOGISTIC) to adjust for the possible confounding effects of maternal age and changes in the maternal serum AFP assay method over time. The maternal age and assay-time variables were entered into the model as continuous variables. An interaction term was created as the cross product of the categoric variables gestational age and maternal serum AFP.

Results

Table I presents the mean maternal serum AFP levels (in multiples of the median) and standard deviations for cases and controls. In both groups the means are lower in the middle gestational age group (16.5 to 17.5 weeks). Between the first and second gestational age intervals, the mean multiples of the median decreases more in cases than in controls. Between the second and third gestational age intervals, the mean multiples of the median increases more in cases than in controls.

Table II shows the fourfold tables stratified by gestational age and gives the corresponding tests for association. Although the odds ratio (7.25) for the middle interval (16.5 to 17.5 weeks) differs considerably from the other two estimates (3.21 and 2.32, respectively), the difference did not achieve statistical significance in the Breslow-Day test for homogeneity. However, the Breslow-Day method tests for differences in the odds ratios on a multiplicative rather than additive scale.

When the data were entered into the multiple logistic regression model to adjust for the potential confounding effects of maternal age and assay-time, the odds ratios (mean and 95% confidence interval) decreased slightly: 2.66 (1.01 to 6.97), 5.76 (1.25 to 26.51), and

1.16 (0.38 to 3.56) for the three respective gestational age intervals. However, when we included maternal age, assay-time, and the interaction term (GA*MSAFP) in the logistic model, the coefficient for the interaction term approached significance (p = 0.148), which suggests a differential effect across gestational age.

Comment

These retrospective case-control data suggest that serum levels of AFP are most predictive of Down's syndrome between 16.5 and 17.5 weeks and that these levels become less predictive with decreasing or increasing gestational age. The interaction between gestational age and AFP is striking, especially for low AFP levels (Table I). Because our numbers are small and we may have underascertained Down's syndrome pregnancies with normal maternal serum AFP levels, our findings must be verified by a larger data set.

Since we presented our preliminary data,5 Waller et al.9 have analyzed data from the California Maternal Serum Alpha-Fetoprotein Screening Program. They found that the Down's syndrome pregnancies with the lowest maternal serum AFP values occurred between 16 and 17 weeks' gestation. Previous data from Wald and Cuckle¹⁰ suggest a trend toward increasing median maternal serum AFP levels in Down's syndrome pregnancies after 19 weeks' gestation.

The effect of gestational age on the efficiency of maternal serum AFP screening has previously been observed for open neural tube defects.11 The detection rate of open spina bifida by elevated maternal serum AFP levels is greater at 16 to 18 weeks' gestation (88% detection) than from 19 to 21 weeks' gestation (54% detection).

If our findings are confirmed, there are several implications: (1) Down's syndrome screening by maternal serum AFP is best done at 16 to 18 weeks' gestation rather than at earlier or later gestational ages, which may be another reason that some Down's syndrome pregnancies are undetected by maternal serum AFP screening.8 (2) The practice of reevaluating low maternal serum AFP levels should be discouraged, especially after 18 weeks. (3) Screening programs may need to take gestational age into account for predicting the risk of Down's syndrome. (4) These findings also may effect predictive values of combined screening that uses maternal serum AFP with human chorionic gonadotropin and unconjugated estriol.12

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Twin gestation: Influence of placentation on fetal growth

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To study fetal growth in twin gestation, morphometric autopsy data of 52 midgestation twin pairs who were stillborn or who died \leq 24 hours after birth were analyzed. Twins were divided into three groups: (1) monozygotic: diamniotic, monochorionic placenta (n=18); (2) dizygotic: diamniotic, dichorionic placenta, unlike sex (n=12); (3) like-sex: placenta diamniotic, dichorionic in 63.6%, unknown in 36.4% (n=22). The monozygotic group had a significantly higher rate of growth discordance, which was defined as a \geq 20% difference in body weight (monozygotic 72.2%, dizygotic 16.7%, like-sex 0%), and polyhydramnios (monozygotic 50%, dizygotic 0%, like-sex 9.1%). Organ weight z scores for body weight and brain weight standards were calculated for the smaller and larger of each twin pair. In the monozygotic group highly significant z scores were obtained for brain weight in the smaller twin (z=2.71, p=0.003, body weight standards) and heart weight in the larger twin (body weight standards, z=3.87, p<0.001; brain weight standards, z=3.64, p<0.001). We conclude that monozygotic twins with diamniotic, monochorionic placentation have a high degree of brain-sparing growth restriction in the smaller twin and cardiac hyperplasia in the larger twin, most likely caused by hemodynamic inequalities. (AM J OBSTET GYNECOL 1991;165:1394-401.)

Key words: Twin gestation, fetal growth, growth discordance, cardiac hyperplasia, intrauterine growth retardation, twin transfusion syndrome

The factors that determine fetal growth are numerous and affect the common pathway of delivery of nutrients and oxygen to the fetus. In singleton gestation these factors include the nutritional state of the mother, blood flow to the uterus, placental size, and placental transport capabilities. Additionally, intrinsic fetal genetic factors may influence fetal processing of delivered nutrients.

In multiple gestation additional factors influence fetal growth. Fetuses may merely be cohabitants of the uterus with separate placentas and fetal circulations, or they may share placentally delivered fuels via vascular connections between fetal circulations. The high degree of twin body weight discordance in monochorionic twins¹ suggests that shared fetal circulations may influence fetal growth. Clinically, inequality of shared fetal circulations is responsible for twin transfusion syndrome, which results in high fetal morbidity and mortality.

Our goal in this study was to assess the influence of vascular connections on fetal growth in twin gestation. This information might help explain the poorly understood phenomenon of twin transfusion syndrome.

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Material and methods

Autopsy records of midgestation twin pairs either delivered at or referred for examination by the University of Michigan from 1974 to 1990 were reviewed. Excluded from review were pairs of twins with chromosomal abnormalities or major malformations in one or both fetuses. Twin pairs in which fetuses were macerated or died >24 hours after birth were also excluded to avoid inaccurate organ weights caused by autolytic degeneration or iatrogenic events in the nursery.

Twins were divided into three groups on the basis of placentation and fetal sex: (1) monozygotic: diamniotic, monochorionic placenta, like-sex; (2) dizygotic: diamniotic, dichorionic placenta, unlike sex; and (3) like-sex: diamniotic, dichorionic placenta or placental evaluation unavailable.

The total body weight and individual weights for the brain, spleen, thymus, thyroid, adrenal gland, kidney, heart, lung, and liver were measured and analyzed for each group. Crown-heel, crown-rump, arm, leg, and foot lengths, and head circumference were also studied. The intertwin difference in body weight for each twin pair was calculated as the weight difference between the heavier and lighter twin divided by the weight of the heavier twin.

Visceral and somatic measurements in each group were compared by calculation of the z statistic according to the following formula: z = Observed - Expected/SD of expected. The expected measurement and its SD were determined from a normal singleton reference population (n = 727, body weight 50 to 1100 gm) established at the University of Michigan. This

Table I. Antenatal complications of twin pairs

		Monozygotic twin pairs $(n = 18)$ Dizygotic twin pairs $(n = 12)$		Like-sex twin pairs $(n = 22)$		
	No.	%	No.	%	No.	%
Major complication leading to delivery						
Preterm rupture of membranes	8	44.4	6	50.0	8	36.4
Preterm labor	8	44.4	5	41.7	10	45.4
Chorioamnionitis	0 .		1	8.3	1	4.5
Incompetent cervix	0		0		2	9.1
Placenta previa	0		0		1	4.5
Severe preeclampsia	- 1	5.6	0		0	
Pregnancy termination	1	5.6	0		0	
Polyhydramnios	9	50.0*	0		2	9.1

^{*}Significantly higher in monozygotic group (monozygotic versus dizygotic, p=0.003; monozygotic versus like-sex, p=0.005).

Table II. Body weight characteristics of twin pairs

	Monozygotic twin pairs (n = 18)		Dizygotic twin p	Dizygotic twin pairs $(n = 12)$		Like-sex twin pairs $(n = 22)$	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Gestational age (wk)	23.9 ± 2.3	18.1-26.5	21.6 ± 3.5	16.7-26.4	23.1 ± 2.5	17.0-26.2	
Body weight (gm)							
All twins	509 ± 219	153-877	384 ± 236	97-807	503 ± 197	113-926	
Lighter twins	441 ± 211	153-776	360 ± 232	97-746	482 ± 196	113-876	
Heavier twins	578 ± 209	210-877	408 ± 248	124-807	524 ± 200	117-926	
Intertwin difference in body weight (%)	$27.3 \pm 13.8*$	5.2-63.4	13.4 ± 7.5	5.9-29.7	8.3 ± 5.0	0.3-18.1	
Pairs with body weight				•			
discordance >20% (%)	72.2	*	16	.7	C)	

^{*}Significantly higher in monozygotic group (monozygotic vs dizygotic, p = 0.004; monozygotic vs like-sex, p < 0.001).

singleton reference population was used because the gestational age of all of the twin pairs studied was <27 weeks, and twin growth only begins to lag significantly behind singleton growth at >29 weeks.2.3 z Score calculations for visceral weights were performed for both body and brain weight standards; z scores for somatic measurements were done for body weight standards. Additional statistical analysis was performed with the two-tailed Student t test and the Fisher exact test. Linear regressions were tested for statistical significance by analysis of variance.

Results

Fifty-two twin pairs were suitable for study. Eighteen pairs had diamniotic, monochorionic placentas and made up the monozygotic group. Twelve pairs had diamniotic, dichorionic placentas and were of unlike sex; they were thus placed in the dizygotic group. The like-sex group consisted of 14 like-sex twin pairs with diamniotic, dichorionic placentas and eight like-sex pairs with unknown placental configuration that may have contained monozygotic twins with diamniotic, dichorionic placentation, dizygotic twins, and even monochorionic, monozygotic twins whose placental configuration was unavailable to us.

Antenatal complications leading to preterm delivery were similar in all three groups (Table I). The major reasons for delivery of these midtrimester twin pairs were premature labor or preterm rupture of membranes with subsequent spontaneous labor or induction. One twin pair in the monozygotic group was from a patient who chose to terminate the pregnancy because of the acute onset of severe polyhydramnios. An antenatal or intrapartum diagnosis of polyhydramnios was made significantly more often in the monozygotic group. Nine (50%) cases in that group had overt polyhydramnios; in three additional cases increased amniotic fluid was noted on ultrasonographic examination (total of 12 polyhydramnios cases, 66.7%). No twin pair in the dizygotic group and only two pairs in the likesex group had polyhydramnios.

The gestational age and mean body weight of all twins in the study groups were comparable (Table II); however, the mean body weight of the smaller and larger twins within the highly discordant monozygotic group differed by 137 gm. Among the three groups, mean intertwin difference in body weight was significantly greater in the monozygotic group (27.3%) than in the dizygotic group (13.4%, p = 0.004) and in the like-sex group (8.3%, p < 0.001). Expressed differ1396 Pridjian, Nugent, and Barr

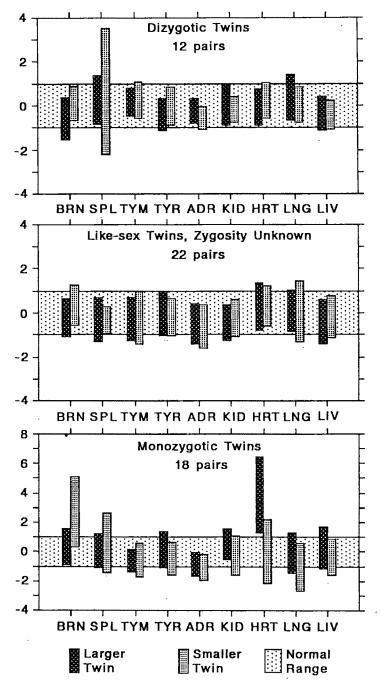


Fig. 1. z Scores (mean \pm SD) of organ weights (calculated for body weight standards) of the larger and smaller twin of each group. Organs studied were brain (BRN), spleen (SPL), thymus (TYM), thyroid (TYR), adrenal gland (ADR), kidney (KID), heart (HRT), lung (LNG), and liver (LIV). Weights of paired organs are combined. Significant mean z scores were found only for brain weight of smaller twins of monozygotic group (z=2.71, p=0.003) and in heart weight of larger twins of monozygotic group (z=3.87, p<0.001).

ently, 72.2% of the monozygotic twin pairs had a body weight discordance of >20%, which was significantly higher than the discordance rate in the dizygotic (16.7%) and like-sex (0%) groups.

The monozygotic (monochorionic) group had high degrees of both body and individual organ weight dis-

cordance. The most dramatic finding was an increased heart size in the larger twins of the monozygotic group. The heart weights of the larger twins were an average of 3.87 SD (p < 0.001) above the normal expected heart weight for body weight (Fig. 1, lower panel), and 3.64 SD (p < 0.001) above the normal expected heart weight

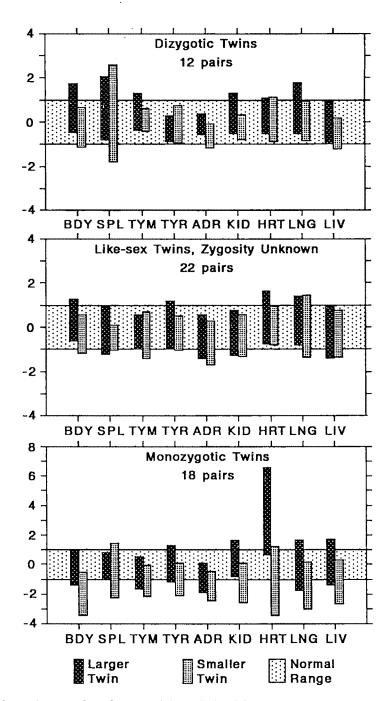


Fig. 2. z Scores (mean ± SD) of organ weights (calculated for brain weight standards) of the larger and smaller twin of each group. Organs studied were brain (BRN), spleen (SPL), thymus (TYM), thyroid (TYR), adrenal gland (ADR), kidney (KID), heart (HRT), lung (LNG), and liver (LIV). Weights of paired organs are combined. Significant mean z scores were found only for body weight in smaller twins of monozygotic group (z = -1.98, p = 0.024) and heart weight in larger twins of monozygotic group (z = 3.64, p < 0.001).

for brain weight (Fig. 2, lower panel). Additionally, the smaller twins had remarkably heavier brains for body weight. The brain weight of the smaller twins was an average of 2.71 SD (p = 0.003) above the expected brain weight for body weight (Fig. 1, lower panel). Conversely, the smaller twins of the monozygotic group had

lighter body weight for brain weight (z = -1.98,p = 0.024; Fig. 2, lower panel).

Individual organs in the smaller twins of the monozygotic group were also somewhat undergrown. Mean z scores for thymus, thyroid, adrenal, kidney, heart, lung, and liver in the smaller twins were appropriate

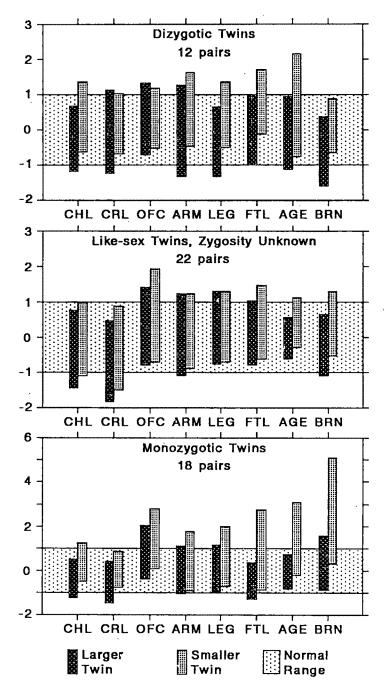


Fig. 3. z Scores (mean ± SD) of somatic measurements of the larger and smaller twin of each group studied. Measurements performed included crown-heel length (CHL), crown-rump length (CRL), head circumference (OFC), and arm length (ARM), leg length (LEG), foot lengths (FTL), gestational age (AGE), and brain weight (BRN).

for their body weight (Fig. 1, lower panel) but were modestly decreased for their brain weight (all z scores at least <-1.00, brain weight standards, Fig. 2, lower panel).

Mean z scores for individual somatic measurements of the smaller twins of the monozygotic group were within normal range for their lighter body weight (Fig. 3, lower panel) except for head circumference. A modestly high mean z score for head circumference in these smaller twins (z = 1.44, p = 0.075) again suggested brain sparing in this group.

Twin pairs that were not monochorionic (dizygotic and like-sex groups) had normal growth. In both the dizygotic and like-sex groups, the mean weights of all organs for both body and brain weight standards and mean somatic measurements for body weight standards were within the normal range.

Further examination of individual twin pairs of the

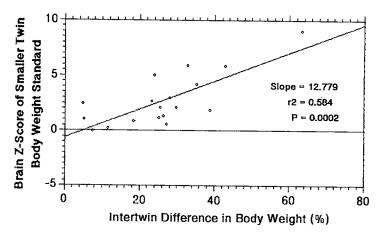


Fig. 4. Correlation of intertwin difference in body weight of individual twin pairs of monozygotic group with degree of brain-sparing growth restriction in smaller twin (graphed as brain z score, body weight standard).

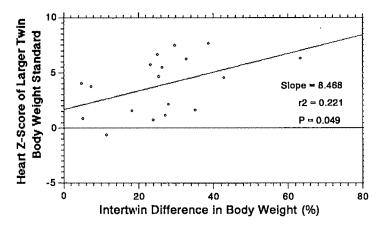


Fig. 5. Correlation of intertwin difference in body weight of individual twin pairs of the monozygotic group with degree of cardiac hyperplasia in larger twin (graphed as heart z score, body weight standard).

monozygotic group revealed that the percentage of body weight discordance between the twins of a pair correlated strongly ($R^2 = 0.584$, p < 0.001) with the degree of brain sparing in the smaller twin (Fig. 4), indicating that asymmetric intrauterine growth retardation of the smaller twin was a feature of this group. The correlation between percentage of body-weight discordance within twin pairs of the monozygotic group and the degree of cardiac hyperplasia in the larger twin (Fig. 5) was not as strong ($R^2 = 0.221$, p = 0.049). Notably, in the monozygotic group the larger twins of two twin pairs with minimal body weight discordance had elevated heart weights (z = 3.73 and 3.97). Correlations in the dizygotic and like-sex groups between degree of body weight discordance and z scores for brain weight or heart weight did not approach statistical significance.

A clinical diagnosis of twin transfusion syndrome was made in 6 of 18 twin pairs of the monochorionic group (33.4%) when growth discordance and acute polyhydramnios were noted. After pathologic evaluation, twin transfusion syndrome was suspected in 15 of 18 cases (83.3%) because of growth discordance, polyhydramnios, and a large heart for body weight in the larger twin. Fetal hematocrit levels were not available.

Comment

Numerous factors affect fetal growth. In this study we evaluated the growth characteristics of prematurely delivered midgestation twins, one group (monozygotic) of whom had placental vascular anastomosis. If carefully inspected, vascular anastomosis connecting fetal circulations can be found in nearly all monochorionic placentas.4 Dichorionic, diamniotic placentation that is either monozygotic or dizygotic in origin is only very rarely associated with fetal vascular communications.5 Thus we considered growth differences in the monozygotic group to be primarily influenced by inequality of fetal blood flow because genetic factors determining growth of twin pairs in this group should be identical. Intertwin differences of growth in the dizygotic group could be attributed to genetic factors, especially those

differences associated with fetal sex, because all twin pairs in this group were of unlike sex. Because the like-sex group was probably heterogeneous in zygosity and placentation, multiple factors could have affected growth in these twins. Surprisingly, this heterogeneous group of like-sex twin pairs had minimal intertwin growth variations. Finally, all three groups of twins were subject to varied, undefined maternal factors that influence growth.

In our study the twin pairs of the monozygotic group had a high degree of intertwin growth discordance and polyhydramnios when compared with the dizygotic or like-sex groups, a finding that agrees with the work of others. ^{1, 6} A large fraction of these twin pairs thus displayed indirect evidence of twin transfusion syndrome. Grennert et al. ⁷ used ultrasonographic measurements to calculate fetal weights and found a greater absolute intrapair weight difference in dizygotic twins. The twin pairs studied were all >28 weeks' gestation and delivered near term. The monozygotic group also contained twins with diamniotic, dichorionic placentation, diluting the differential growth effects of vascular anastomosis.

The proportion of monochorionic twins in our study (35%) was greater than would be expected if compared with the incidence of monochorionic twins in the population (20%).8 Our overrepresentation of monochorionic twins may be caused by either an increased midgestation loss of this type of twin or an increased referral of this type of twin for detailed evaluation.

In body weight-discordant pairs of the monozygotic group, the larger twin was of normal weight and the smaller twin was underweight. Others have reported similar findings by using ultrasonographic estimations of fetal weight in twins carried to near term.9 In our study the smaller twin of the monozygotic group displayed brain-sparing growth restriction. The degree of brain sparing in these twins correlated with the percentage of intertwin body weight difference, indicating that restricted growth of the smaller twin and not overgrowth of the larger twin is responsible for body weight discordance. Four of the body weight-discordant twin pairs of the monozygotic group had brain weight discordance >20% (data not shown), suggesting that some of these fetuses were subject not only to brain-sparing growth restriction but perhaps also to factors that influence growth very early in gestation and result in decreased brain cell number.10

In the smaller twins of the monozygotic group, the majority of the remaining internal organs studied were also undergrown. Except for head circumference, somatic measurements of these twins also corresponded to their lighter weight.

The most dramatic finding in this study was the high degree of cardiac hyperplasia¹¹ in the larger twin of the monozygotic group. In this group an increased heart weight in the larger twin was found in eight of nine twin pairs with overt polyhydramnios. However, the degree of heart weight increase correlated weakly with the degree of body weight discordance. The larger twins of two twin pairs with minimal body weight discordance were found to have large hearts and polyhydramnios; these findings suggest that hemodynamic imbalance between twins may precipitate cardiac hyperplasia before a major growth disturbance is evident.

We found that monochorionic twin pairs have a high degree of brain-sparing growth restriction in the smaller twin and cardiac hyperplasia in the larger twin. We support the theory that twin transfusion syndrome is a condition that begins early in embryonic vascular development. Benirschke12 described the youngest example, a pair of aborted twin embryos measuring 7 and 8 cm (10 weeks' gestation) in which the heart size of the donor twin was half that of the recipient. Further, we suggest that intertwin vascular distribution established early in the embryonic period may control intertwin placental mass distribution. Inequality in placental mass distribution may have an early direct effect on fetal growth, causing significant growth restriction in one fetus. Alternatively, intertwin vascular connections may produce chronic twin transfusion syndrome, in which the larger twin responds with cardiac hyperplasia. Cardiac hyperplasia may be compensatory and certain sets of monochorionic twins may withstand the vascular inequalities throughout gestation (subclinical twin transfusion syndrome). In others, the pumping capabilities of the enlarged heart are exceeded and cardiac failure occurs, producing a more acute picture of twin transfusion syndrome. It is tempting to speculate that when the heart enlarges and fails, delicate pressure-flow characteristics in the placental vasculature are disrupted, exacerbating shunting to the recipient twin.

It is not surprising that the appropriate diagnostic criteria used to detect twin transfusion syndrome in the newborn period are still debated¹³ because the mechanisms leading to acute twin transfusion syndrome are still unclear. Cardiac hyperplasia may initially be a physiologic response to increased circulatory volume, but beyond a certain degree it may play a role in the development of the syndrome. In women with ultrasonographically diagnosed monochorionic twins,¹⁴ the usefulness of echocardiography for the antenatal diagnosis of twin transfusion syndrome should be studied. Longitudinally assessed echocardiographic parameters in monochorionic twins may shed light on the natural history of the syndrome and may be predictive of adverse outcomes in these twin pairs.

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Myocardial necrosis in a newborn after long-term maternal subcutaneous terbutaline infusion for suppression of preterm labor

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We report a case of myocardial necrosis in a newborn after treatment of the mother with long-term subcutaneous terbutaline. No such serious side effects in the fetus have previously been reported. We speculate that this myocardial damage was due to β-sympathomimetic therapy. (AM J OBSTET GYNECOL 1991;165:1401-4.)

Key words: Cardiomyopathy, infant, subcutaneous, β-sympathomimetics

β-Sympathomimetic agents have become accepted as therapy for the suppression of preterm labor. Continuous subcutaneous terbutaline hydrochloride has recently been administered for long-term tocolysis.¹ Long-term (9 weeks) maternal subcutaneous terbutaline infusion has resulted in no maternal or fetal complications.¹ We report a case of an infant in whom dilated cardiomyopathy and myocardial necrosis occurred after long-term, low-dose subcutaneous terbutaline infusion.

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6/1/30688

Case report

A male infant was born at 37 weeks' gestation to a 22-year-old, gravida 2, para 1 woman. The pregnancy was complicated by gestational diabetes and preterm labor. At 25 weeks' gestation continuous, subcutaneous terbutaline infusion (0.5 mg/hr) for treatment of preterm labor was begun. During the final 2 weeks of pregnancy, additional 0.3 mg bolus infusions were added each hour to the baseline infusion.

At birth the baby weighed 2850 gm and appeared normal. However, tachypnea of 80 to 100 breaths/min developed in the infant. No evidence of infection was detected. Chest radiography showed mild cardiomegaly with increased pulmonary vascularity. Tachypnea persisted, and on the fourth day of life a cardiologic evaluation was performed. The heart rate was 145 beats/min, respirations were 60 breaths/min, and blood pressure was 81/58 mm Hg. There was a grade 2/6 systolic murmur at the lower left sternal border

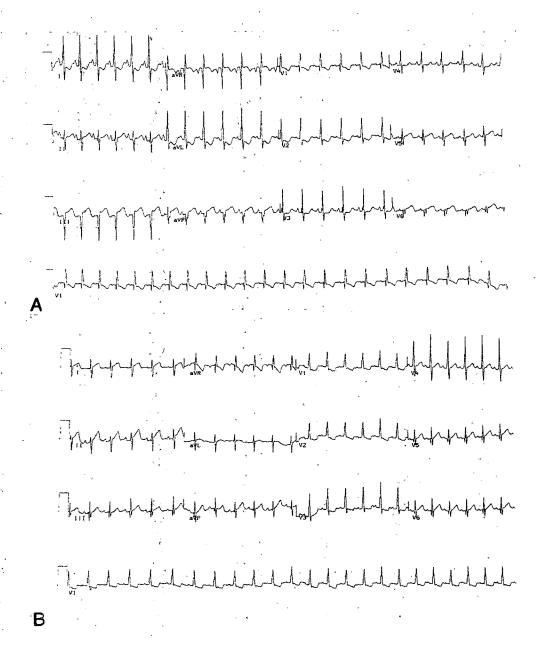


Fig. 1. A, Initial electrocardiogram showing abnormally deep Q waves in inferior leads with ST segment elevation. Reciprocal ST segment depression is seen anteriorly. B, Note small residual Q waves in inferior leads without ST segment changes. This electrocardiogram was done 1 month after initial presentation.

with normal pulses; hepatosplenomegaly was not present. Electrocardiogram showed deep Q waves in aVR, aVF, and III with ST segment elevation (Fig. 1, A). Echocardiogram showed decreased left ventricular shortening fraction of 12% (normal, 32% to 40%) and an enlarged end-diastolic dimension of 2.5 cm (upper normal, 2.0 cm) (Fig. 2, A). Tricuspid regurgitation was present.

Treatment for congestive heart failure was initiated with digoxin 10 mcg/kg/day, furosemide 2 mg/kg/day, and captopril 2 mg/kg/day. The baby improved clin-

ically, and the respiratory rate normalized by the seventh day of life.

Infectious and metabolic causes of cardiomyopathy were excluded. Cardiac catheterization on day 9 showed normal coronary arteries. Right ventricular biopsy showed marked myocardial fiber degeneration and focal bizarre nuclear dysmorphism (Fig. 3), which were independently interpreted as being consistent with myocardial injury caused by catecholamine excess.

The infant recovered rapidly and went home on day 12. At 1 month of age no abnormal findings were pres-

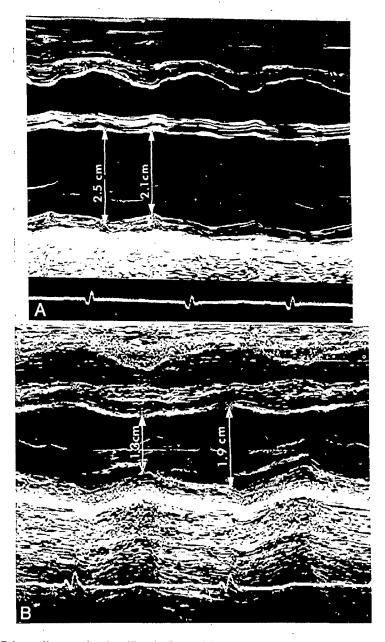


Fig. 2. A, Echocardiogram showing dilated left ventricle with markedly decreased shortening fraction of 12%, compared with B, which shows return of left ventricle to normal dimensions and systolic function.

ent. The electrocardiogram showed a normal sinus rhythm with small residual Q waves in the inferior leads and resolution of ST segment changes (Fig. 1, B). Echocardiogram showed normal left ventricular dimensions and a normal shortening fraction of 32% (Fig. 2, B).

Comment

Subcutaneous β-sympathomimetic infusion is a relatively new form of tocolytic therapy. Initial clinical trials have suggested that subcutaneous infusion has a longer duration of tocolysis than does intermittent high-dose oral therapy. All "β2-selective" agents, however, have significant β-sympathomimetic cardiostimulatory side effects.

Bohm and Adler² performed autopsies on the hearts of 25 infants whose mothers had received intravenous or oral "β₂-specific" sympathomimetics for tocolysis for up to 12 weeks. Deaths were attributed to complications of prematurity. Histologic examination showed myocardial necrosis, fatty degeneration, and nuclear polyploidization.2

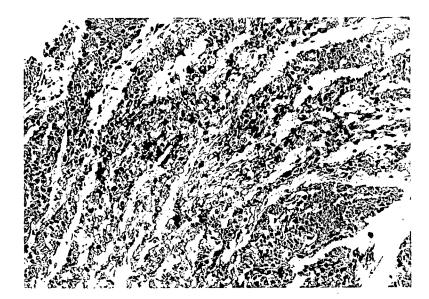


Fig. 3. Severe vascular degeneration of myofibers with early clumping of sarcoplasm. Few enlarged myofiber nuclei are seen in subendomyocardium. (Hematoxylin and eosin. Original magnification \times 200.)

Various forms of myocardial injury by β -sympathomimetics have been proposed, such as excessive catecholamine cardiac stimulation, diminished circulatory pressure, increased metabolic demand, and calcium influx into the myoplasm of cardiac cells.

Although a causative relationship between cardiomyopathy and β -adrenergic receptor therapy cannot be proved in this case, circumstantial evidence includes prolonged exposure to β -adrenergic receptor agonist therapy, poor ventricular contractility with no structural anomaly, and histologic demonstration of myocardial necrosis identical to that described with catecholamine excess.

Delivery systems for continuous long-term β -adrenergic stimulation must be carefully evaluated for ad-

verse fetal receptor effects. Continuous low-dose terbutaline infusion may maximize tocolytic effectiveness by minimizing β -adrenergic receptor down regulation. However, such receptor down regulation may be protective to the fetal myocardium. The fetal cardiac effects of continuous long-term β -adrenergic receptor tocolysis need further evaluation.

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Vasopressin pack for treatment of bleeding after myoma resection

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In 17 women with refractory bleeding after myoma resection a dilute vasopressin pack was applied. Twenty units of vasopressin was diluted with 30 ml normal saline solution. A 1-inch new gauze pack was soaked in the dilute vasopressin and packed into the uteri of patients with bleeding from the beds of resected submucous myomas. The pack was left in place for no more than an hour. In none of the cases was there bleeding after the removal of the pack nor were there any side effects that could be attributed to the vasopressin. (AM J OBSTET GYNECOL 1991;165:1405-7.)

Key words: Vasopressin pack, bleeding

Heavy bleeding from the bed of either a completely or a partially resected submucous myoma during or after operative hysteroscopy can at times be difficult to control. One of the more common methods for managing this problem is the insertion of an intrauterine balloon or a Foley catheter with a 30 ml balloon and then inflating the balloon with as much fluid as possible to tamponade the bleeding. The catheter is left in place for as short a time as 2 to 3 hours or as long as 24 hours.1-3 When this technique was used in several patients, the degree and duration of pain were impressive. Because of the stimulatory effects of vasopressin on the smooth muscle of the uterus and blood vessels and the known benefit of compression on bleeding, a dilute vasopressin-soaked pack was placed in the uterus to assess the utility of this method to control bleeding in a series of patients who had undergone partial or complete resection of submucous myomas.

Material and methods

The study population comprised 17 of 150 women between the ages of 25 and 50. This group of patients had surgery between February 1988 and July 1990. Of this group, five were Nulliparous and the remaining had from one to four term pregnancies. All complained of heavy menstrual bleeding with two thirds requiring oral iron for maintenance of hemoglobin between 8 and 10 gm. In the remaining patients the hemoglobin ranged between 10 and 12 gm with oral iron. In all cases the menstrual bleeding interfered with normal activities. None of the patients had evidence of any

coagulation disorders or evidence of thyroid dysfunction. Hysterectomy was strongly suggested in all of the women in spite of the fact that several desired children. All patients had requested alternative means of therapy to control the bleeding.

More than half of the patients had had a curettage performed within 12 months of the operative procedure, and in none of the cases was the submucous myoma detected nor was the bleeding controlled by curettage. Within 6 months of the operation all patients had received some type of hormone therapy, either oral contraceptives or various progestins, without benefit.

All patients underwent office video hysteroscopy with dilute dextran 70 (Hyskon, Pharmacia Laboratories, Piscataway, N.J.) as the distending medium to establish the diagnosis. Biopsies of suspicious areas were performed, and the specimens were used to establish ovulation.

In five patients the objective was to resect the myoma only to control the bleeding, and in four of these fertility was the major issue. In the remaining 12 patients the plan was to resect the myoma and then perform roller-ball endometrial ablation to markedly reduce or stop the menstrual flow.

All patients received some type of preoperative medication to suppress ovulation and thin the uterine lining. In seven patients danazol (Danocrine, Winthrop Pharmaceuticals, New York), 800 mg a day for 6 weeks, was used; in three patients medroxyprogesterone acetate (Depo-Provera, Upjohn Co., Kalamazoo), 400 mg 4 weeks before the planned surgery, was given; and in the remaining seven patients medroxyprogesterone (Provera, Upjohn Co.), 10 mg twice a day for 6 weeks, was used.

A 2 to 3 mm laminaria tent was placed into the cervix and through the internal cervical os 24 hours before surgery in all patients, and all were started on a regimen of doxycycline hyclate (Vibramycin, Pfizer, Parsippany,

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N.J.), 100 mg twice a day. All were given a prescription for hydrocodone bitartrate (Vicodin, Knoll Pharmaceuticals, Whippany, N.J.) for preoperative and postoperative pain control. On the day of surgery the patients were given a general anesthetic, the laminaria tent was removed, and the patients were prepped and draped for the procedure. A 3% sorbitol solution was used as the irrigating medium, and an Olympus 27 Fr. dual-channel irrigating resectoscope (Olympus Corp., Lake Success, N.Y.) was used in performing the procedures. In those patients in whom resection only was performed, a wire loop electrode was used with a cutting current of 120 W blend 2. In those individuals who were undergoing both resection and ablation, a rollerball electrode was attached to the instrument after resection of the myoma, and the endometrium was coagulated at 100 W. At the end of the operative procedure, the irrigating fluid was stopped, and the resected base was examined. If brisk bleeding was observed, attempts were made to control the bleeding by coagulating the base with either a wire loop or rollerball electrode. In 17 cases the bleeding was not sufficiently controlled as determined by bleeding that filled the vagina over a 30-second time period. Packing was performed with a 1-inch new gauze cotton packing that had been soaked with a dilute vasopressin solution, i.e., 20 U vasopressin diluted with 30 ml injectable normal saline solution. The moistened gauze was firmly packed into the uterine cavity with either a packing forceps or long smooth finger forceps. As much packing as possible that could be firmly placed in the cavity was used. This was performed quite easily because in all patients the cervix had been softened and dilated with the laminaria tent. In one patient the entire pack (60 inches) was placed in the cavity, but in most instances from one fourth to one half of the pack was used. The patients were awakened from anesthesia and taken to the recovery room. Vital signs were observed every 15 minutes for the next 2 hours. The pack was removed within 1 hour after surgery, and the patients were ambulated. If bleeding was insignificant, the patient was discharged within 1 hour. All patients were seen at 6 weeks and 6 months after surgery.

In the five patients who were undergoing resection only, the entire tumor was resected in four cases and in the remaining instance it was felt that at least two thirds of tumor had been removed. In those patients who were undergoing resection and ablation, the myomas were resected completely in six and in the remaining at least two thirds or more before ablation.

Results

In all cases the dilute vasopressin pack appeared to be successful in controlling bleeding after the operative procedure. In none of the patients was there any significant bleeding through the pack for the first hour after surgery. None of the patients had any significant degree of bleeding, i.e., no more than one soaked pad per hour after the pack was removed. In fact, in all patients there was only light staining of the perineal pad.

After discharge none of the patients complained of any significant bleeding during the immediate post-operative period. At 6 weeks and 6 months they were carefully queried as to the degree of bleeding, and in none of the cases was it significant. In those patients who had only resection of a myoma, the menses returned to normal, according to the patients. In two patients, pregnancy was achieved within 6 months after surgery, and one has been delivered of a healthy term infant; the remaining patient is still pregnant. In those individuals who had had resection and ablation, all had no bleeding or only spotting at 6 weeks and 6 months after therapy.

All of the patients complained of some degree of pain in the immediate postoperative period, but narcotics were required to relieve the pain only in half. Once the pack was removed, none noted further pain. Although all patients had received prescriptions for analgesics for the postoperative period, only two used the medication, for 1 day only.

The irrigating fluid that was used during surgery varied between 8000 and 56,000 ml, and in each case a fluid balance was within accepted guidelines, i.e., fluid output versus fluid input difference of ≤1000 ml.⁴

All patients had careful monitoring of blood pressure and vital signs while they were in the recovery room, and in none of the cases was there any evidence of tachycardia, hypertension, or hypotension.

Comment

The results of this study indicate that a dilute vasopressin pack is a safe and effective way to control bleeding from the bed of a resected submucous myoma that cannot be controlled with cauterization. Because of reports of problems with injecting even dilute vasopressin,⁵ it was decided not to inject the tissues directly but to deliver the vasopressin to the tissues by a packing technique. The duration the pack was left in the uterus was arbitrary. Because the patients wanted to go home, it was initially decided that the pack would be removed within an hour in the first six patients. Because they did so well, this practice was continued and has been continued to date. It has not been necessary to repack because of bleeding.

Compression for control of bleeding has been used for centuries. Intrauterine packing for postabortion and postpartum bleeding is controversial. Packing was used in these cases both to deliver the vasopressin into the uterus and to apply topical compression. Whether a pack alone or vasopressin alone would be just as effective remains to be determined and is the subject of additional studies.

Vasopressin is primarly an antidiuretic hormone. The drug also causes active contraction of virtually all smooth muscles in the body, including blood vessels, intestines, and uterus. Injected vasopressin has been used to control bleeding with operative hysteroscopy.² Because of reports of death with injected vasopressin,⁵ it was elected not to inject the tissue in these patients but rather to deliver the vasopressin by pack. The mechanism of action in this study is probably twofold. The pack provides control of bleeding by compression, but when the packing material contains vasopressin, the uterus undergoes further contraction that adds to the benefits of the pack. A tertiary effect may be in the vessels of the uterus.

None of the patients had exhibited any sign of infection, but all had been placed on a regimen of antibiotics before surgery. Moreover, none of the patients had any evidence of the cardiovascular changes that are reported with vasopressin.

It appears, from this evaluation in 17 patients who underwent partial or complete resection of submucous

myomas with refractory bleeding, that a dilute vasopressin pack is an effective and safe way to control such bleeding. The pack can be removed within a short time, thereby permitting an early discharge. It is urged, however, that when patients undergo operative hysteroscopic procedures they should remain relatively inactive for about 3 weeks, as we have known of bleeding in some patients who exercised vigorously during the first 21 days after surgery.⁴

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Severe preeclampsia in the second trimester: Recurrence risk and long-term prognosis

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A total of 125 women with severe preeclampsia that developed in the second trimester underwent follow-up for an average of 5.4 years. Seventeen women had no further pregnancies and 108 had 169 subsequent pregnancies: 59 (35%) were normotensive and 110 (65%) were complicated by preeclampsia (32% of these developing in the second trimester, 32% at 28 to 36 weeks, and 36% at 37 to 40 weeks). Overall, 21% of subsequent pregnancies were complicated by severe preeclampsia in the second trimester. Forty-four patients (35%) had chronic hypertension, the highest incidence being in those with recurrent severe preeclampsia in the second trimester and the lowest in those with only normotensive subsequent pregnancies (67% vs 4%, p < 0.0001). Long-term maternal complications included two maternal deaths and two other patients with end-stage renal disease requiring dialysis. We conclude that these women are at increased risk for repeat preeclampsia, particularly in the second trimester, and are at increased risk for chronic hypertension and maternal mortality and morbidity. (AM J OBSTET GYNECOL 1991;165:1408-12.)

Key words: Preeclampsia, second trimester, recurrence, chronic hypertension

Severe preeclampsia developing in the second trimester is a rare complication of pregnancy. These pregnancies are usually associated with increased perinatal mortality and morbidity. ¹⁻³ In addition, such pregnancies are associated with significant maternal morbidity: pulmonary edema, disseminated intravascular coagulopathy, hypertensive encephalopathy, convulsions, and acute renal failure. ^{1, 2, 4}

Counseling of the patient whose pregnancy is complicated by severe preeclampsia during the second trimester is a difficult task for the obstetrician, because there are no data regarding risk of recurrence and long-term maternal outcome. We previously reported that women having severe preeclampsia-eclampsia in their first pregnancy are at increased risk for having preeclampsia in subsequent pregnancies and at increased risk for chronic hypertension later in life. However, very few patients studied in that report had severe preeclampsia in the second trimester. The purpose of this study is to report subsequent pregnancy outcome and remote maternal prognosis in 125 women with severe preeclampsia that developed in the second trimester.

Material and methods

The study population consisted of 125 women who had severe preeclampsia during the second trimester

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6/1/30373

(18 to 27 weeks' gestation). The diagnosis and management in these cases were previously described. 1,2 All patients were delivered during the index pregnancy at the E.H. Crump Women's Hospital and Perinatal Center, Memphis. Patients with preexisting diabetes mellitus, connective tissue disease, or sickle cell disease and those with inadequate medical follow-up were excluded. Inclusion criteria included adequate medical follow-up for a minimum of 2 years after delivery of the index pregnancy.

Pregnancy outcome in subsequent pregnancies was reviewed with regard to gestational age at time of delivery, incidence of preeclampsia, and perinatal outcome. Only pregnancies delivered beyond the first trimester are included in this analysis. The diagnosis of chronic hypertension was based on the presence of documented hypertension (either before index pregnancy or on follow-up) for which the patient received antihypertensive therapy. Maternal blood pressure measurements were performed with the patient in the sitting position after 5 minutes of quiet rest; the arm used was in a roughly horizontal position at heart level. The fifth (disappearance) Korotkoff sound was used to determine the diastolic blood pressure.

All patient records were carefully and extensively reviewed by B.M.S. In the majority of cases (90%) the patients were seen and followed up by B.M.S. In other cases hospital records and the records of other medical providers were accepted only if they were determined to be adequate. Maternal blood pressure recordings were obtained at 6 to 8 weeks post partum and then once every year.

Data analysis was done with the use of χ^2 and odds ratios; p < 0.05 was considered significant.

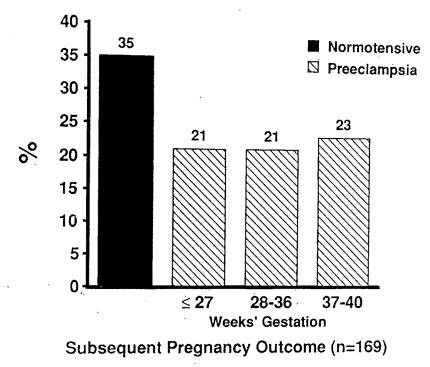


Fig. 1. Pregnancy outcome in 108 women who had subsequent pregnancies: 35% were normotensive and 65% were complicated by preeclampsia.

Results

Table I summarizes the clinical findings in the index pregnancy among the 125 women studied. The 125 women underwent follow-up for an average of 5.44 years (range, 2 to 12). Seventeen women had no further pregnancies, and 108 had 169 subsequent pregnancies (range, one to four per patient). In 59 (35%) of these pregnancies the women were normotensive, whereas 110 (65%) were complicated by preeclampsia. Of those complicated by preeclampsia, it developed in the second trimester in 35 (32%), at 28 to 36 weeks in 35 (32%), and at 37 to 40 weeks in 40 (36%). Fig. 1 summarizes the overall incidence of preeclampsia in subsequent pregnancies according to gestational age at the time of onset. Overall, 21% of subsequent pregnancies were complicated by severe preeclampsia in the second trimester.

The 169 subsequent pregnancies resulted in 170 births (one set of twins). There were 22 stillbirths and six neonatal deaths, for an overall perinatal loss of 16.5%. Eleven (6.5%) of the pregnancies were complicated by abruptio placentae, and 39 (23%) were complicated by fetal growth retardation (birth weight <10th percentile for gestational age). Table II summarizes the pregnancy outcome in subsequent pregnancies according to whether the women were normotensive or had preeclampsia. There were two perinatal deaths in the normotensive group; one was a stillbirth at 32 weeks' gestation (normal findings on autopsy), and one was a neonatal death after preterm delivery at 24 weeks' gestation due to premature rup-

Table I. Clinical findings at time of index pregnancy

Maternal age (yr, mean \pm SD)	23.6 ± 4.9
Maternal age <25 yr	
No.	80
%.	64
Nulliparous	
No.	101
%	80
Black	
No.	83
%	66
Gestational age at onset (wk, mean ± SD)	25.3 ± 1.7
Gestational age at delivery (wk, mean ± SD)	26.8 ± 1.8
Birth weight (gm, mean ± SD)	701 ± 173

ture of membranes. In addition, the majority of poor pregnancy outcomes in the preeclamptic group were in those who had repeat second-trimester preeclampsia.

A total of 44 patients had chronic hypertension; 21 of these women had chronic hypertension before the index pregnancy (one of them also had glomerulonephritis). The other 23 patients had evidence of chronic hypertension on follow-up. The average time interval from delivery to the development of hypertension in these patients was 6.1 years (range, 1 to 11).

Seven (41%) of the 17 women with no subsequent pregnancies had chronic hypertension; six of these had chronic hypertension before the index pregnancy and the other patients had chronic hypertension on follow-

Intrauterine growth Perinatal loss Abruptio placentae retardation % No. No. · No. 6.8 1.7 Normotensive 3.4Preeclampsia 35 31.5 26 23.410 9.1≤27 wk* 20 23 63.9 5 14.3 55.1 28-36 wk 11 31.4 3 8.6 3 8.6 37-40 wk 10 5.0

Table II. Pregnancy outcome in subsequent pregnancies

Table III. Presence of chronic hypertension according to subsequent pregnancy outcome (N = 108 women)

	.No. of	Chronic hypertension		
Outcome	No. of patients	No.	%	
Normo- tensive	25	1	.4*	
Preeclampsia	. 83	36	43	
≤27 wk̂	27	18	67*	
28-36 wk	30	12	40	
37-40 wk	26	6	23	

^{*}p < 0.0001; odds ratio, 48:1.

Table IV. Development of chronic hypertension on follow-up according to subsequent pregnancy outcome (N = 93*)

	. N C	Chronic hypertension		
Outcome	No. of patients	No.	%	
Normo- tensive	25	1	4†	
Preeclampsia	68	21	31	
≤27 wk	20	11	55†	
28-36 wk	25	7	28	
37-40 wk	23	3	19	

^{*}Excludes 15 patients with previous chronic hypertension. $\dagger p < 0.0002$; odds ratio, 29.3:1.

up. On the other hand, 37 (34%) of the 108 with subsequent pregnancies had chronic hypertension, 15 before the index pregnancy, whereas the other 22 patients had chronic hypertension on follow-up.

A total of 93 patients without evidence of chronic hypertension before the index pregnancy had subsequent pregnancies on follow-up. Eighty-nine were normotensive, and four had persistent elevations of blood pressure 6 to 8 weeks after delivery of the index pregnancy. The latter four patients had chronic hypertension that developed after 1 to 3 years of follow-up. The remaining 89 patients who were normotensive post par-

tum had a total of 130 subsequent pregnancies. In 58 (45%) of these pregnancies the women were normotensive, whereas 72 (55%) pregnancies were complicated by preeclampsia. Among these, preeclampsia developed in the second trimester in 20 (28%), at 28 to 36 weeks in 25 (35%) and at 37 to 40 weeks in 27 (37%).

Table III describes the frequency of chronic hypertension, according to subsequent pregnancy outcome among the 108 women studied. The incidence of chronic hypertension was significantly higher in those women, who had subsequent preeclampsia than in those who were normotensive in subsequent pregnancies (43% vs 4%, p < 0.0001; odds ratio, 18.4:1). The highest incidence of chronic hypertension was in those women with recurrent severe preeclampsia in the second trimester (67%).

The data were subsequently analyzed after the 15 women who had chronic hypertension before the index pregnancy were excluded. Table IV describes the incidence of chronic hypertension diagnosed on follow-up, according to outcome in subsequent pregnancies. Again, the incidence of chronic hypertension was significantly higher in women who had subsequent pre-eclampsia as compared with those with only normotensive subsequent pregnancies (31% vs 4%, p < 0.005; odds ratio, 10.7:1). In addition, the highest incidence of chronic hypertension was among women with recurrent severe preeclampsia in the second trimester (55%).

In four patients serious maternal complications developed during the follow-up period; two of them died and two patients had end-stage renal disease necessitating dialysis. All four patients had long-standing chronic hypertension, and three of the four had the index pregnancy complicated by abruptio placentae, fetal death, disseminated intravascular coagulation, and acute renal failure. One patient had acute renal failure caused by bilateral cortical necrosis immediately after delivery of the index pregnancy. This patient had no subsequent pregnancies. The other three patients had subsequent pregnancies that were complicated by severe preeclampsia in the second trimester; one of them died of a cerebrovascular accident 5 years after

^{*}n = 36 (one set of twins).

the index pregnancy (3 months after the recurrent second-trimester preeclampsia). One patient had three consecutive pregnancies complicated by preeclampsia in the second trimester (two of which also were complicated by acute renal failure). She required dialysis 7 years after the index pregnancy and eventually died at 9 years 5 months after the first episode. In the remaining patient with preexisting glomerulonephritis end-stage renal disease developed about 21/2 years after the index pregnancy and is currently on dialysis after 5 years of follow-up.

Comment

Two recent studies revealed that women who have preeclampsia in their first pregnancy are at increased risk for having preeclampsia in subsequent pregnancies.5.6 In addition, both studies found that the risk of having preeclampsia in subsequent pregnancies is related to the time of onset of preeclampsia during the first pregnancy. However, neither of these studies described the risks for women with severe preeclampsia that develops in the second trimester.

The results of this study show that women with severe preeclampsia that develops in the second trimester are at very high risk for severe preeclampsia in subsequent pregnancies. In such women 65% of all subsequent pregnancies were complicated by preeclampsia. It is important to emphasize that 21% of all subsequent pregnancies were complicated by severe preeclampsia in the second trimester and 21% were complicated by severe preeclampsia at 28 to 36 weeks' gestation. Thus these patients should be informed of the high likelihood of severe preeclampsia and poor perinatal outcome in subsequent pregnancies. Women considering pregnancy should be instructed to seek prenatal care early in the first trimester. In addition, they should be seen more frequently, starting in the second trimester, so that the onset of hypertension can be detected as early as possible. Moreover, such patients might be good candidates for receiving low-dose aspirin during pregnancy to prevent the onset of early preeclampsia.7

Patients with severe preeclampsia that develops remote from term are at increased risk of chronic hypertension and undiagnosed underlying renal disease.5,6,8,9 Ihle et al.8 studied renal function at 6 weeks to 6 months post partum in 84 patients with severe preeclampsia that developed at 24 to 36 weeks' gestation. Maternal evaluation included a 24-hour urine study, urine microscopy, intravenous pyelography, and renal biopsy when appropriate. The authors found a high incidence of renal disease and essential chronic hypertension (90%). In two thirds of these patients the lesion was glomerulonephritis, mostly of the immunoglobulin A variety. They concluded that such women usually have underlying renal disease that should be evaluated after delivery. In contrast, Lin et al.9 reported on the pregnancy outcome and prognosis in 157 women in whom the etiology of hypertensive complications of pregnancy was determined by postpartum renal biopsies. The authors found that most of the women (62%) who were delivered before 37 weeks' gestation had the renal lesion of preeclampsia.

The results of this study show that patients having severe preeclampsia in the second trimester have a significantly high incidence of chronic hypertension. This incidence is significantly higher in those having subsequent hypertensive pregnancies. In addition, the incidence is highest in those with severe preeclampsia that again develops in the second trimester. On the other hand, patients who are normotensive in subsequent pregnancies have an extremely low incidence of chronic hypertension on follow-up (Table IV). This information may be useful for the counseling of such patients about subsequent medical follow-up.

It is important to emphasize that women having severe preeclampsia in the second trimester are at increased risk for long-term maternal morbidity or for mortality. Two of these women died; one death was due tò a cerebrovascular accident and one was a result of end-stage renal disease. In addition, end-stage renal disease necessitating dialysis developed in two other patients. All four serious complications developed in women with long-standing, preexisting chronic hypertension. Interestingly, only three of these four patients had subsequent pregnancies, and all subsequent pregnancies were complicated by severe preeclampsia in the second trimester. Thus such patients should be informed about these risks and should be counseled regarding close medical follow-up in the future.

In summary, patients with severe preeclampsia that develops in the second trimester should be considered at increased risk for severe preeclampsia in subsequent pregnancies and at increased risk for chronic hypertension. In addition, women with recurrent severe preeclampsia that develops in the second trimester are at increased risk for maternal mortality and morbidity later in life.

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A case report of prostaglandin E₂ termination of pregnancy complicated by Cushing's syndrome

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A case of Cushing's syndrome caused by an adrenal adenoma seen at 10 weeks' gestation is described. The pregnancy was terminated at 18 weeks' gestation, representing the first reported case of therapeutic termination of pregnancy in Cushing's syndrome with prostaglandin E₂ vaginal suppositories. (AM J OBSTET GYNECOL 1991;165:1412-3.)

Key words: Cushing's syndrome in pregnancy, hypertension in pregnancy

A case of Cushing's syndrome associated with an adrenal adenoma at 10 weeks' gestation is reported.

Case report

A 28-year-old black woman, gravida 3, para 2, was referred to University Hospital for termination of pregnancy at 13 weeks' gestation with a presumptive diagnosis of Cushing's syndrome. The patient was first admitted to a community hospital at 10 weeks' gestation for evaluation of hypertension. At this time blood pressure was 206/150 mm Hg with otherwise unremarkable findings at physical examination. She denied any history of hypertension or serious medical illness and had last been examined by a physician 7 months earlier. Menses had been regular to date. Laboratory parameters were obtained; hemoglobin value, serum electrolyte levels, serum creatinine level, creatinine clearance value, and thyroid and liver function test results were normal. One-hour glucose tolerance testing with a 50 gm load was elevated at 176 mg/dl. Twenty-four-hour urinary metanephrine levels were normal. Evaluation of the adrenal axis revealed an evening serum cortisol level of 33.1 µg/dl, 24-hour urinary free cortisol level of 1000 µg, and a 24-hour urinary 17-hydroxycorticosteroid value of 13.1 mg. These values were interpreted as normal for pregnancy. The patient was discharged after antihypertensive therapy was adequately established.

She was seen by the visiting obstetric consultant in

She was seen by the visiting obstetric consultant in the outpatient clinic at 13 weeks' gestation. The patient appeared lethargic and admitted to easy fatigability. Blood pressure was poorly controlled at 180/120 mm Hg. Physical examination was unchanged except for the presence of moon facies and purple striae over the abdomen and upper and lower extremities. The abdomen was protuberant. Presumptive diagnosis of Cushing's syndrome was made on the basis of the findings at physical examination and the previously determined adrenal studies. The patient indicated at this time her wish to terminate the pregnancy, and she was referred to the tertiary care center for continuing evaluation and pregnancy termination.

The patient was lost to follow-up until 17 weeks' gestation, at which time she was admitted to University Hospital. Biochemical evaluation revealed frank diabetes with fasting blood glucose levels of 150 to 200 mg/dl. Repeat evaluation of adrenal function confirmed the previously noted elevation: Diurnal variation of serum cortisol was abolished with a morning level of 71.8 mg/dl and an evening level of 59.6 mg/dl. Twenty-four-hour urinary free cortisol level

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was 1035 µg. A low-dose 48-hour dexamethasone suppression test failed to suppress production of urinary free cortisol.

Before completion of endocrine evaluation, the patient threatened to leave the hospital against medical advice if the pregnancy was not terminated. Pregnancy termination was accomplished uneventfully with the use of intracervical laminaria and prostaglandin E2 vaginal suppositories. Fentanyl epidural analgesia was provided early in the course of labor induction to minimize the sympathetic nervous system's hypertensive response to pain. She was delivered of a normal-appearing but small-for-gestational age fetus weighing 70 gm; the placenta was histologically normal but correspondingly small at 35 gm. Radiographic evaluation of the pituitary and adrenal glands with computerized axial tomography was undertaken because of the patient's demonstrated lack of compliance; it revealed a 2 × 3 cm right adrenal nodule. When this information was used as a confrontational device, the patient agreed to further biochemical evaluation, which was performed 8 days post partum. It revealed that the serum corticotropin level was <20 pg/ml. Sequential low-dose 48hour and high-dose 48-hour dexamethasone suppression tests failed to suppress urinary free cortisol and 17-hydroxycorticosteroid levels. The patient underwent right adrenal exploration, and right adrenalectomy yielded a 3 × 2 × 1.7 cm benign cortical adenoma. Her postoperative course was uncomplicated, and she was discharged on a regimen of clonidine and prednisone replacement therapy. She has subsequently done well with complete resolution of clinical symptoms and biochemical derangements.

Comment

The occurrence of pregnancy in a case of untreated Cushing's syndrome is rare because of the high incidence of ovulatory disturbances associated with the disorder.1 As illustrated in this report, diagnosis is difficult because of the development of a physiologic hypercortisolism in normal pregnancy and the affective changes

in the cushingoid patient's mental status, which lead to noncompliance with medical evaluation.

Maternal morbidity associated with Cushing's syndrome is excessive, with hypertension and glucose intolerance being the most common complications.2 Congestive heart failure, proximal myopathy, depression, and poor wound healing also are noteworthy. Death has occurred in three instances.2 Pregnancy outcomes are associated with high perinatal morbidity and mortality rates, with prematurity rates approximating 60%.2 In addition, fetal intrauterine growth retardation has been noted in one-fourth to one-third of all cases (unpublished observations). Perinatal mortality is equally divided between neonatal and intrauterine deaths and thus may represent the combination of complications of prematurity and the effect of a hostile intrauterine environment created by maternal disease (unpublished observations).

Therapy for Cushing's syndrome in pregnancy should be directed toward control of hypercortisolism either through chemotherapy or by surgical extirpation, because maternal and perinatal outcomes are unacceptably poor with supportive therapy alone. Great care should be taken to correctly identify the endocrine source of the disease, be it pituitary, adrenal, or ectopic production of corticotropin. Finally, Cushing's syndrome and pregnancy are rarely coincident, but the diagnosis should not be overlooked in the pregnant woman with appropriate signs and symptoms.

We acknowledge the help of Drs. George Elias and Thomas Grace in the surgical management of this case.

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Bleeding after intravascular transfusion: Experimental and clinical observations

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Characteristics of postpuncture bleeding of umbilical vessels were evaluated with an in vitro cord perfusion model and in vivo by ultrasonographic observation of bleeding duration after intravascular transfusion. Ultrasonographic determination of blood loss in vitro was very sensitive (0.01 ml/sec). In vitro blood loss varied directly with perfusion rate, but there were wide variations between cord specimens. Observed clinical bleeding occurred in 43% of cases; the duration of bleeding varied by vessel punctured, needle size, and fetal platelet count. The combined in vitro and clinical data help define the range of duration of bleeding and the probable volume of loss. (AM J OBSTET GYNECOL 1991;165:1414-8.)

Key words: Intravascular transfusion, fetal bleeding

Ultrasonography-guided percutaneous umbilical vessel puncture, first introduced by Daffos et al.,¹ has become a standard method for fetal diagnosis and therapy. Fetal bleeding from the puncture site is commonly observed by contemporary ultrasonographic methods, but little is known about the duration, volume, and character of this bleeding. The purpose of this study was threefold: to determine in vitro the volume threshold of blood loss that may be detected by ultrasonography, to determine in vitro blood loss per unit time after 22-gauge needle puncture of the umbilical vein and its relationship to cord perfusion rate, and to determine by clinical observation the duration and character of bleeding after umbilical vessel puncture done for intravascular transfusion.

Material and methods

The minimal blood loss that could be detected by real-time ultrasonography was determined by an in vitro method. A glass water bath was filled with tepid tap water and allowed to settle until all visible bubbles dissipated. A 3.5 MHz linear-array transducer was aligned to the water bath and a 22-gauge spinal needle was placed in the water bath such that the tip was submerged approximately 4 cm and visualized by ultrasonography. A 1 ml plastic syringe was filled with freshly collected citrated whole blood, and the needle shaft and tip were imaged continuously (Acuson model 128). With minimal infusion pressures, the smallest vol-

ume of blood that could be reproducibly detected was recorded.

The relationship between umbilical vein blood flow volume and blood loss was determined by an in vitro method. Umbilical cords were collected immediately after delivery in 10 women who were >36 weeks' gestation at delivery. All collected specimens were fresh, were at least 15 cm in length, and were judged to be normal on gross inspection. The umbilical vein was immediately flushed with heparinized saline solution until all visible clots were eliminated, and then catheters of maximal permissible diameter were attached to the distal and proximal ends of the umbilical vein and secured. A constant-volume perfusion pump, attached to a recirculation circuit, perfused the umbilical vein with citrated whole human blood (hematocrit 48%).

In separate experiments the relationship between infusion volumes and umbilical venous pressure was determined; the maximal venous perfusion pressure used in this study was 12 torr. After a constant-infusion loop was established, the umbilical vein was punctured as close to the perpendicular as possible with a 22-gauge needle. Care was taken not to lacerate the umbilical vein at the time of puncture. The loop of cord with the puncture site was placed gently over a funnel and graduated beaker, and the blood loss for precisely 1 minute was collected as the cord was perfused. The collected blood volume was measured with a calibrated pipette and validated with dye dilution; both yielded an error of <0.1 ml. Blood loss from the punctured umbilical vein was measured across a range of constant perfusion rates ranging from 50 to 200 ml/min. The sequence of perfusion volumes was varied randomly and all experiments were repeated in duplicate. Subsequently, the umbilical cords were frozen with the catheters in situ. After freezing at -27° C for at least 24 hours, cords were thawed and the experiments were repeated.

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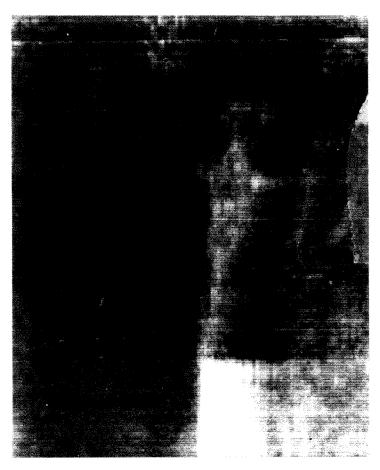


Fig. 1. Water bath image of infusion of only 0.025 ml of blood through a 22-gauge needle (bright linear echoes at left), producing turbulence.

Clinical observations of the duration of visible intraamniotic bleeding after umbilical vessel puncture were made with 3.5 MHz transducer and an ultrasonographic imaging system (Acuson model 128). Observations of bleeding time were made in 264 consecutive intravascular transfusions done because of severe fetal alloimmune disease in 72 fetuses, 23 of whom were hydropic at first transfusion. The gestational age at procedure ranged from 19 to 35 weeks. The duration of postpuncture bleeding was defined as the time between immediate removal of the needle from the fetal vessel and the inability to detect blood loss from the puncture site. In addition to duration of bleeding, qualitative characteristics of the bleeding were noted. Delivery follow-up was available on all fetuses.

Parametric statistical methods were used to assess data and included regression analysis and Student t test for unequal variance. Statistical significance was designed as a p value ≤ 0.05 .

Results

With an in vitro sequential graduated trial method, the minimal discrimination for blood loss from the tip of a 22-gauge needle by an ultrasonographic method

was 0.01 ml/sec. Above this minimal detection threshold, no direct relationship between the rate of blood loss from the needle tip and the ultrasonographic characteristics could be identified (Fig. 1).

The rate of blood loss from punctured perfused umbilical vein segments in vitro was related in a direct and linear fashion to the umbilical vein perfusion rate (r = 0.873, p < 0.01) (Fig. 2). In duplicate studies on the same vein at different perfusion rates, the mean variation between repeat experiments was 0.6 ml/min. In combined observations blood loss (mean ± SEM) from the puncture site ranged from as low as 2.99 ± 0.43 ml at a 50 ml/min perfusion rate to as high as 6.77 ± 0.64 at a perfusion volume of 200 ml/min (Table I). Blood loss varied widely from one fresh cord segment to another, ranging from 0.3 to 8.3 ml/min at the 50 ml perfusion rate, 0.5 to 10.13 ml/min at the 100 ml perfusion rate, 1.3 to 14.08 ml/min at the 150 ml perfusion rate, and 1.68 to 18.4 ml/min at the 200 ml perfusion rate. Within individual cord specimens, blood loss increased with incremental increases in infusion rate in 52 of 60 observations (86.6%), was not measurably different in five observations (8.3%), and decreased in three observations (5%). Standardization

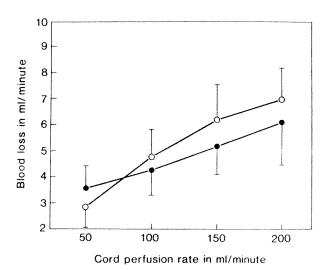


Fig. 2. Relationship between blood loss per 22-gauge puncture of umbilical vein and rate of perfusion of vein. In vitro experiments were done in 10 fresh cords (open circles) and after freezing (closed circles). Blood loss did not vary between fresh or frozen cords and bore a direct and significant relationship to perfusion volume (r = 0.873, p < 0.01).

of blood loss by using the percent change per incremental increase in perfusion rate yielded a significant inverse linear relationship between percentage loss and perfusion rate (r = 0.757, p < 0.01).

Clinical observations were made in vivo immediately after needle removal in 264 intravascular transfusion procedures (packed cell transfusion, hematocrit ≥85%, average transfusion volume of 50 ml/kg estimated fetal weight). In 152 instances, the cord vessel puncture was on the placental side of the vessel and intraamniotic bleeding was not observed. In 112 cases (43.2%) cord puncture was transamniotic, and back-bleeding occurred, permitting determination of intraamniotic bleeding time and character. Data from these 112 procedures were analyzed.

Postpuncture bleeding characteristics were assessed both qualitatively and quantitatively.

Qualitative characteristics. The characteristics of postpuncture bleeding appear to vary by the vessel punctured. Venous bleeding was characterized within the amniotic space by an immediate showering of echodense material that gravitated toward the dependent portion of the uterus. The ultrasonographic echos were most evident and dramatic at immediate needle removal and gradually diminished in a decrescendo pattern until disappearance. It was common to observe a continued slow and sometimes intermittent showering preceding complete cessation of visible bleeding. Arterial bleeding was distinctly different on ultrasonographic scanning. Needle removal was associated with a plume of blood that often projected up, struck the

chorionic plate, and showered back. The characteristic pluming effect seemed to persist unabated for a period of time and then ceased abruptly. Evidence of slow bleeding from arterial puncture sites was infrequent.

Quantitative analysis. Eighty-five umbilical vessel puncture procedures were done with a 22-gauge needle in 72 venipunctures and 13 umbilical artery punctures. The mean observed bleeding time for 22-gauge umbilical vessel puncture was 137 seconds (range 5 to 720 seconds) (Table II). Bleeding time varied significantly by the vessel punctured; the mean bleeding time was 144 seconds (range 5 to 450 seconds) after venipuncture (n = 72) and 95 seconds (range 5 to 720 seconds) after arterial puncture (n = 13) (p < 0.05).

Twenty-seven umbilical vessel punctures were done with a 20-gauge needle, 22 of which were venipunctures and 5 were umbilical artery punctures. The mean bleeding time after 20-gauge umbilical vessel puncture was 92 seconds (range 5 to 300 seconds). The mean duration of venous bleeding in 22 instances was 102 seconds (range 20 to 350 seconds), and in 5 cases of arterial puncture mean duration of bleeding was 48 seconds (range 5 to 300 seconds).

Fetal platelet count was determined in 110 of 112 procedures and was related to the duration of post-puncture bleeding. Fetal platelet count was normal in all 27 procedures done with a 20-gauge needle. In 83 of the 85 procedures done with a 22-gauge needle, fetal platelet count was available. In 75 instances the fetal platelet count was normal (platelet count $\geq 50,000/\text{ml}$) (range 80 to 400,000/ml); the mean bleeding time in these cases was 123 seconds (range 5-400 seconds). In eight procedures the platelet count was abnormal (6000/ml) to 49000/ml); the mean bleeding time in fetuses with thrombocytopenia was 373 seconds (range 140 to 720 seconds). These differences were significant $(p \leq 05)$.

In nine fetuses a clinical diagnosis of fetal exsanguination was made on the basis of a combination of findings, including prolonged bleeding (≥300 seconds) of a quantitative nature judged to be severe. All fetuses had gross abnormalities of fetal heart rate and loss of other biophysical variables. Two of these fetuses (31.6 and 32.5 weeks, respectively) were delivered by prompt cesarean section, were severely anemic and volume depleted at birth, but responded to resuscitation and survived. The remaining 7 fetuses were <32 weeks at diagnosis of fetal exsanguination (age range 21 to 31 weeks). In three of these fetuses immediate prompt repeat intravascular transfusion was done, including in one fetus with intracardiac transfusion, and all responded promptly to volume replacement and survived. In the remaining four cases (all <24 weeks' gestation), immediate vascular access could not be rees-

Table I. Blood loss at different constant perfusion rates

	Blood loss (mean \pm SEM, ml/min)						
	50 ml/min	100 ml/min	150 ml/min	200 ml/min			
	perfusion	perfusion	perfusion	perfusion			
Trial 1 $(n = 10)$	2.84 ± 0.37 3.52 ± 0.41 2.99 ± 0.43	5.56 ± 0.43	6.16 ± 0.62	7.52 ± 0.43			
Trial 2 $(n = 10)$		4.26 ± 0.52	5.16 ± 0.34	6.09 ± 0.71			
Combined trials $(N = 20)$		$4.56 \pm 0.47*$	5.67 ± 0.53*	6.77 ± 0.64*			
Change $(N = 20)$ $(\%)$		100 ± 12.3	36.6 ± 9.4*	23 ± 7.8†			

^{*}Compared with next lowest perfusion rate, p < 0.01.

tablished and all fetuses died promptly. Death was attributed to severe hypovolemia and cardiovascular collapse.

Comment

From our clinical observations we noted that all procedures in which umbilical vessel puncture occurred distal to the chorionic plate and placental substance (43% of total procedures), bleeding was detected by real-time ultrasonographic methods. The in vitro experimental observations offer insight to the nature of this bleeding.

The detection of egress of blood from a 22-gauge needle by dynamic ultrasonography was surprisingly sensitive; in these studies the minimal volume threshold for detection was at least as low as 0.01 ml/sec. Whereas unclotted whole blood is more echo dense than amniotic fluid or saline solution, these media in a static state cannot be differentiated by ultrasonography. In the dynamic state these minor differences in echo density become amplified to the observer, and blood is easily identified by its specular echo density pattern as it falls down through the water medium. From these titrated observations it is evident that detection of bleeding by ultrasonography is sufficiently sensitive to be of clinical usefulness in timing the duration of the event. Whereas qualitatively larger-volume bleeding tends to produce wider and more spectacular echo-dense patterns than bleeding of lesser volume, these differences are highly subjective and not possible to quantitate. Thus it is disappointing to note that from these in vitro observations the rate and volume of bleeding after vessel puncture cannot be quantified by ultrasonography to an accuracy level that is clinically useful.

In our experience most umbilical vessel punctures for diagnostic or therapeutic reasons are done at gestational ages at or below 30 weeks. Direct measurements of umbilical blood flow in the ovine and porcine fetus are reported as 160 to 2001 and 160 to 170 ml/min, respectively,2 and by indirect ultrasonographic calculation methods to be 110 to 120 ml/min in the human fetus.3 The constant volume perfusion rate of the um-

Table II. Comparison of bleeding times with 22-gauge needle versus 20-gauge needle

		Postpuncture bleeding time (sec)			
Category	No.	Mean	Range		
22-Gauge needle					
Total cases	85	137	5-720		
Venous puncture	72	144	5-450		
Arterial puncture	13	95*	5-720		
Normal platelets	75	123	5-400		
Thrombocytopenia	8	375†	140-720		
20-Gauge needle					
Total cases	27	92‡	5-300		
Venous puncture	22	102	20-350		
Arterial puncture	5	48*	5-350		

^{*}p < 0.05, as compared with venous bleeding time.

bilical vein in vitro used in this study would approximate the expected flow observed in human fetuses with weights from 500 to 1750 gm, the usual weight range of fetuses subjected to in utero umbilical vessel puncture.

Major differences between the in vitro model in this study and the clinical circumstance must exist. In the cord perfusion model bleeding from the puncture site was observed to be relatively constant. In contrast, qualitative observation of postpuncture bleeding in the human fetus demonstrated a decrescendo pattern of loss, evident in observation of both venipuncture and arterial puncture and most dramatic in the latter. Occlusion of the puncture site in vivo may be the result of platelet and fibrin accumulation, local vessel spasm at the site of puncture, or both. The in vitro studies demonstrated no difference in bleeding rate between fresh and frozen cords, which may indicate that vessel wall changes are of lesser importance than local coagulation. The clinical observation of significantly prolonged bleeding in human fetuses with thrombocytopenia may suggest that platelets are important in limiting postpuncture bleeding.

[†]Compared with next lowest perfusion rate, p < 0.05.

 $[\]dagger p < 0.05$, as compared with normal platelet count.

p < 0.05, as compared with 22-gauge needle puncture.

The pure physics model would predict that within a constant pressure range egress from a perpendicular puncture of fixed aperture would vary in a direct linear manner with flow rate. The observation of the in vitro cord perfusion model approximates the theoretic model (Fig. 2). However, whereas an increase in perfusion was generally associated with an increased loss in individual experiments, wide variation between cord specimens was observed (Table I). The explanation for this variation is not clear because aperture size, perfusion rates, and perfusion pressures were well controlled. It is possible that the holes produced by the needle were not uniform or that vein friability resulted in enlargement of the hole when subjected to perfusion pressure. If such were the case, it was not visible by inspection of the puncture site. Whatever the reason for the individual variation, the phenomenon is important in interpreting probable blood loss in vivo. Total fetal blood loss after vessel puncture, the salient clinical point, must be the product of rate and duration of bleeding. Since the threshold for detection of bleeding by ultrasonography is so low, it follows that measurement of duration of bleeding is likely to be very accurate. In the clinical part of this study postpuncture bleeding was noted also to vary widely; mean duration of bleeding was significantly greater with venipuncture, was significantly prolonged in fetuses with disease severe enough to cause thrombocytopenia, and varied significantly between a 20- and 22-gauge aperture. Given the inability to quantitate blood loss by ultrasonography, the individual variability in loss per cord specimen, the variability in duration of bleeding, and the lack of precise information on cord perfusion rate and pressure, in vivo calculation of actual blood loss is impossible. It is also clear from the clinical observations in the study that life-threatening fetal exsanguination can result from postpuncture bleeding.

In spite of these limitations of measurement error, combining the in vitro and in vivo observation may offer some insight into the usual expected loss and the extremes that may occur. For example, using mean venipuncture bleeding time (144 seconds or 2.4 minutes) as the basis for comparison, the average expected blood loss across the perfusion range of 50 to 200 ml/min would be 5 to 6 ml at the 50 ml/min rate to 12 to 13 ml at the 200 ml/min perfusion rate. Average loss in the fetus is likely to be less than these estimates in view of the observed decrescendo pattern of bleeding. With

the assumption of an average fetal blood volume of 125 ml/kg and a direct relationship between fetal weight and umbilical blood flow,³ average losses of these magnitudes would be <5% of total fetal blood volume. In contrast, with maximal observed loss figures from the in vitro observations, in vivo loss for the fetus could be as high as 19 to 20 ml at the 50 ml perfusion rate and as high as 44 to 45 ml at the 200 ml perfusion rate; these losses could represent up to 20% to 25% of total blood volume. Such loss may cause fetal anemia but is unlikely to cause irreversible hypovolemia.

The duration of fetal bleeding also must be a critical component of hemorrhagic fetal compromise after vessel puncture. In the clinical observation in this study the maximal duration of bleeding was 720 seconds (12 minutes). Bleeding of this duration could yield an average net blood loss of 28 to 30 ml at 50 ml/min perfusion to 62 to 63 ml at 200 ml/min perfusion, representing ≥50% of fetal blood volume. Combining prolonged bleeding time with maximal cord loss observation could yield fetal blood loss of up to 90 ml at the 50 ml/min perfusion rate up to 220 ml at the 200 ml/min perfusion rate; bleeding of the magnitude would certinaly lead to hypovolemic collapse.

These combined clinical and experimental data offer some insight into the extent of fetal bleeding after vessel puncture and may be of some value in clinical application. It is evident that in the average case blood loss is likely to be minimal and not to exceed 5% to 10% of total blood volume. It is equally evident that prolonged bleeding, either spontaneous or in the presence of thrombocytopenia, may result in exclusive fetal loss up to as much as 30% to 90% of total fetal blood volume. The exact determination of loss would appear to be beyond conventional ultrasonographic measurement capabilities. Planned studies of amniotic fluid blood content or serial changes in fetal hematocrit are planned and may shed additional light on this common procedural complication.

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Recombinant 8 syndrome: The pool of Hispanic pericentric inversion 8 carriers expands numerically and geographically

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Recombinant 8 syndrome is a well-established syndrome with mental and developmental retardation and usually severe cardiac anomalies. A carrier parent will produce affected offspring in 6% of pregnancies and carrier offspring in 53% of such pregnancies. Four New Mexican kindreds ascertained by the discovery of four apparently unrelated probands with cytogenetically confirmed recombinant 8 syndrome were studied. We found that (1) recombinant 8 syndrome will soon no longer be confined to New Mexico and southern Colorado, (2) the number of persons at risk may be higher than previously considered, and (3) through proper pedigree techniques and increased professional education, most carriers can be identified. (AM J OBSTET GYNECOL 1991;165:1419-22.)

Key words: Recombinant 8 syndrome, chromosomal aberration, mental retardation

Rec(8),dupq,inv(8)(p23q22) is a chromosomal aberration causing a well-established dysmorphic syndrome with mental and developmental retardation and usually severe cardiac anomalies.1-3

The first case of a child born with the characteristic duplication-deletion of chromosome 8 was described by Fujimoto et al.1 Since then, many more cases have been discovered and described. The most recent report was by Smith et al.4 at the University of Colorado.

All of the children were from the southwestern United States with Hispanic ancestry, and all were diagnosed as having congenital heart disease and dysmorphic features. Chromosomal analyses showed dupq,inv(8)(p23q22), an unbalanced recombinant that arises through meiotic recombination of a normal chromosome 8 and chromosome 8 with a pericentric inversion, in one of the affected child's parents.⁴⁻³

Carriers of the pericentric inv(8) appear to have no phenotypic abnormalities. The recombinant 8 chromosome results in a dysmorphic syndrome characterized by congenital heart disease, ranging from a small ventricular septal defect to classic tetralogy of Fallot, developmental and mental retardation, hirsutism, cleft lip and palate, raised nasal bridge, flat nose, elongated philtrum, prominent lateral folds along each side of the nose, hypertelorism, short wide neck, short stature, and fifth-finger camptodactyly3 (Fig. 1). Through the work

Fig. 1. Male child with recombinant 8 syndrome showing square face, low frontal hairline, long palpebral fissures, broad high nasal bridge, upturned nose, and square earlobes.

of collaborators at the University of New Mexico and the University of Colorado,24 the number of carrier

Hispanic families ascertained has increased. The risk

of an inversion carrier passing on the inv(8) chromo-

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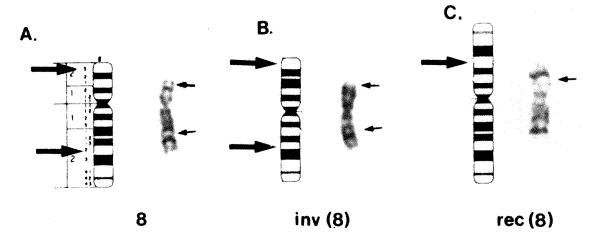


Fig. 2. Idiogram of chromosome appears on left of each pair; actual banded chromosomes on right. *Arrows* denote breakpoints. **A,** Normal chromosome 8; **B,** carrier, inv(8)(p23q22); **C,** affected, rec(8),dupq,inv(8)(p23q22).

some to a child is approximately 53% (59% from mothers, 42% from fathers) while that of rec(8) is calculated as 6.2%. On the basis of these data, a prospective investigation to gather additional data on the existing pedigrees both vertically (increased generations) and horizontally (increased numbers of individuals) was performed. The intent was to test older people in the pedigrees in the hope of linking older generations and to test younger people in the pedigrees who were planning to start families. Those shown to be inversion carriers would be offered genetic counseling and prenatal diagnosis.

Material and methods

Four New Mexican kindreds ascertained by the discovery of four apparently unrelated probands with cytogenetically verified recombinant 8 syndrome were evaluated. Family pedigrees were expanded by obtaining information during home visits to family members in Albuquerque and several northern New Mexico villages and from patients visiting the fetal diagnosis and therapy clinic at the University of New Mexico Hospital. Cytogenetic analysis was performed by standard Giemsa-trypsin banding methods of peripheral blood lymphocytes and amniotic fluid cell cultures. ^{3, 6}

Peripheral lymphocytes obtained by venipuncture from 23 patients and amniotic fluid from one patient were analyzed cytogenetically. Diagnostic procedures were performed at the University of New Mexico Women's Specialty Clinic. Statistical evaluation was performed by χ^2 analysis as a test for goodness of fit of the expected risks.

Results

From July 1988 through June 1990 we evaluated 28 individuals. Of these, 4 (14%) had the recombinant 8

syndrome and 24 had normal phenotypes. Cytogenetic analysis of the 24 phenotypically normal family members showed that 11 (46%) carried the pericentric inversion of chromosome 8 and that 13 (54%) had normal karyotypes (Fig. 2, *A*, *B*, and *C*). Prenatal cytogenetic diagnosis offered to one carrier couple at risk showed a 46,XX fetus.

The χ^2 analysis of the frequency of pericentric inv(8) in this group of individuals was not significantly different (1 df resulted in a value of 0.495 with a p value of 0.48). The χ^2 analysis for the risk of rec(8),dupq,inv(8)(p23q22) probands during this study resulted in a value of 2.283 with a p value of 0.130, which is not statistically different from that of previous studies.

With these four pedigrees added, the total number of kindreds in New Mexico is 12. Two pedigrees were traced back six generations linking these two kindreds at the sixth-generation ancestor level (Fig. 3). One other kindred arose in a tiny northern New Mexico village where three previous families have been linked.

Two thirds of these four kindreds' progeny live away from New Mexico: Texas, Arizona, California, Oregon, Illinois, Maryland, Tennessee, and Virginia (Fig. 4).

Comment

Chromosomal inversions fall into two groups, pericentric and paracentric. In pericentric inversion the two breaks that originally preceded the inversion are in opposite arms.⁷ In paracentric inversion they are in the same arm. Before chromosomal banding techniques, pericentric inversions were discovered by the altered position of the centromere, relative to the distance to either telomere. Chromosomes vary with which arm inversion is likely to occur; for instance, inversion in chromosome 9 accounts for 40% of all inversions.⁴ The

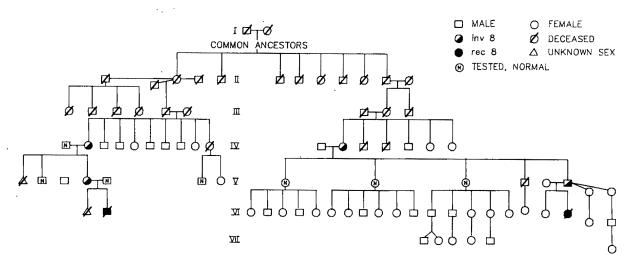


Fig. 3. Pedigree linking two families. Note carrier parents in generations IV and V, discovered by parental testing of probands. Deceased carriers are linked by noting relationships of obligate carriers.

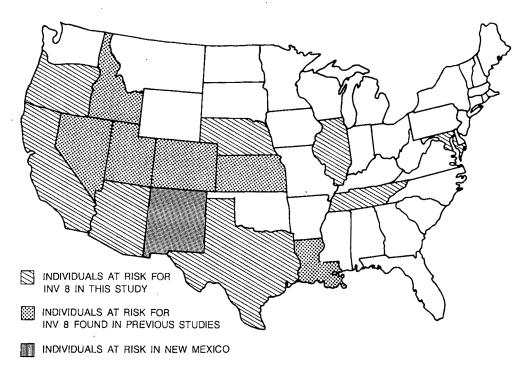


Fig. 4. States in which inversion 8 kindreds have been identified.

location of the break points occurs along the length of the chromosome in a nonrandom fashion. During meiosis, short heterozygous inversions remain unpaired. A large inverted segment usually forms a loop to pair with its homologue, or it may pair straight, leaving the now nonhomologous ends unpaired. Crossing over in a pericentric inversion leads to a deletion and duplication, a phenomenon called aneusomie de recombinaison by French authors.8 The larger the inversion, the more likely the recombination duplication-deletion will occur. Both duplication and deletion may give rise to abnormal but viable offspring. It is surprising how

few of the inversions in man seem to lead to reproductive abnormalities, either by the birth of recombinant offspring or by partial sterility.7-9

The prevalence of recombinant 8 syndrome in the southwestern United States is continually growing. There are affected children in all southwestern states, as well as known carriers and other family members in most of the rest of the United States. Until recently, the families who migrated from New Mexico and Colorado have all been of Hispanic origin, but through intermarriage, there are now members of other groups at risk as well.4 This study shows that the number of 1422 Izquierdo et al.

people currently at risk for carrying inv(8)(p23q22) and those having children with severe congenital anomalies is greater than previously expected. The actual numbers of carriers and people at risk in New Mexico are no less than those calculated. Still, many branches of the pedigrees are incomplete, and some go back eight generations. We linked two apparently unrelated families at a sixth-ancestor level, supporting the theory of a common founder at least 350 years ago in the southwestern United States.3 There is a single report of a Hispanic child with recombinant 8 syndrome and rec(8) chromosome in the Argentinean literature.10 This finding suggests a founder earlier than our current estimation, perhaps from Spain. We have been unable to obtain a pedigree on this family. Given that the risk for inheritance of the pericentric inversion is approximately 50% and that there is no effect on the health or fertility of these carriers, we can expect a geometric increase in the number of both carriers and affected children in the next decade.

The large number of carriers and people at risk in these families and the many as yet incomplete branches in these pedigrees point to a need for follow-up of these families, completion of the pedigrees, testing of possible carriers, and provision of genetic counseling and follow-up for concerned parents. The basic dysmorphic features of the affected children should be diagnosable by high-resolution ultrasonography.11 These features include congenital heart defects, cleft lip and palate, elongated philtrum, and hypertelorism. Physicians working in the southwestern United States who evaluate patients with a history of mental retardation and heart defects should include recombinant 8 syndrome in their differential diagnosis. In fact, at the University of New Mexico Medical Center, congenital heart defect and southwestern United States Hispanic ancestry are an indication for cytogenetic analysis.

Further work with this problem should include ex-

panding the existing family pedigrees to identify additional members and risks of carrying the inversion, performing chromosome analysis on the large number of individuals who we have identified to be at risk, ascertaining additional children in known or new families with recombinant 8 syndrome, gaining more knowledge concerning the proper treatment and the most effective educational approaches for affected children, and educating physicians and the general public about this syndrome.

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Subchorionic hematomas and the presence of autoantibodies

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Five cases of subchorionic hematoma detected by ultrasonography in patients with threatened abortion are presented. Three of these subjects had antinuclear antibodies, and the remaining two subjects had anticardiolipin antibodies. We recommend that patients with subchorionic hematomas be tested for autoantibodies regardless of their obstetric history. (AM J OBSTET GYNECOL 1991;165:1423-4.)

Key words: Subchorionic hematoma, autoantibodies, anticardiolipin antibodies, antinuclear antibodies, habitual abortion

Lupus and allied disorders, as well as anticardiolipin antibodies, are associated with poor fetal outcome. Recently, a high prevalence of antinuclear antibody—positive serum was demonstrated in patients with recurrent pregnancy losses. The incidence of anticardiolipin antibody in the general obstetric population is 1% to 2%; it is approximately 40% in patients with a poor obstetric history.

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In this manuscript we describe five cases of subchorionic hematomas that were associated with autoantibodies. The patients presented with vaginal bleeding in the late first trimester or early second trimester of pregnancy. The clinical details are summarized in Table I.

Three of these patients had had one or more spontaneous abortions. One of the remaining two patients was primigravid; the other had had an induced abortion. In all these patients subchorionic hematomas were detected by ultrasonography that was performed because of threatened abortion. Anticardiolipin or antinuclear antibodies were also detected.

The presence of vaginal bleeding during early pregnancy is associated with a 10% to 15% risk of spontaneous abortion. The incidence of a subchorionic he-

Table I. Clinical features of patients with subchorionic hematomas

Patient and obstetric history	Current pregnancy	Antibody status	Treatment	Pregnancy outcome
para 1, previous spontaneous abortion at 20 wk associated with premature cervi- cal dilatation	Bleeding associated with sub- chorionic hematoma noted at 17 wk		Cerclage at 13 wk, prednisone 30 mg orally on alternate days at 10 wk	Normal spontaneous vaginal de- livery at term, male infant
28 yr old, gravida 4, para 0-0-3-0, spontaneous abortions at 24 wk and 12 wk	Bleeding and subchorionic hematoma noted at 15 wk	ANA 1:160	Aspirin 75 mg daily begun at 11 wk	Cesarean section for breech at term, female infant
35 yr old, gravida 6, para 1-0-5-1, vol- untary termina- tion of pregnancy spontaneous abortions at 12 and 6 wk	Bleeding at 13 wk, subchorionic hematoma noted at 15 wk	ANA 1:40	Aspirin 75 mg daily begun at 9 wk, prednisone 30 mg daily begun at 15 wk	Normal spontaneous vaginal de- livery at term, female infant
24 yr old, gravida 1, para 1, one vol- untary termina- tion of pregnancy	Spotting at 6 wk, subchorionic hematoma noted at 6 wk 5 days and disappeared at 13 wk	ACL Ab (+)	Expectant	Patient at 14 wk of pregnancy
	Bleeding at 11 wk, subchorionic hematoma persisted	ACL Ab (+)	Aspirin 75 mg daily begun at 13 wk	Neonatal death at 24.8 wk

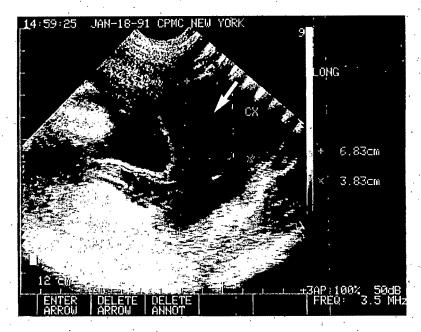


Fig. 1. Arrow points to subchorionic hematoma.

matoma detected by ultrasonography in women with threatened abortions varies widely (11% to 62%). Several studies have demonstrated that patients who have a subchorionic hematoma are more likely to abort (13% to 40%). Pedersen et al. Suggested that the high rate of spontaneous abortions (40%) observed in subjects with subchorionic hematomas may have been due to a patient selection bias (most of those patients had a poor obstetric history). This theory is supported in a study by Mandruzzato et al., who found that 50% of women who presented with an intrauterine hematoma had had a previous spontaneous abortion. In any case, the etiologic and epidemiologic factors of subchorionic hematomata remain unclear.

We propose that the presence of autoantibodies may be an etiologic factor. These antibodies may increase the tendency of platelets to aggregate, which leads to thrombosis and/or vasculitis and thereby to an increased likelihood of a subchorionic hematoma. Although subplacental bleeding in patients with lupus has been reported, subchorionic hematomas have not been described.

We recommend that patients with threatened abortion and subchorionic hematomas detected by ultrasonography (Fig. 1) be evaluated for autoantibodies, regardless of obstetric history. Those patients with a poor obstetric history and persistent hematomas should be treated aggressively with low-dose aspirin and/or corticosteroid medications.

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The dimension of chaos in the fetal heart rate

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Variability in the fetal heart rate is known to be a sign of fetal well-being, and yet the origins of the variations remain unclear. This study incorporated the nonlinear analytic techniques of phase-space reconstruction and dimensional analysis to 12 normal heart rate tracings obtained from fetal scalp electrodes of fetuses in labor. Phase-space attractors were constructed with the method of time delays and showed characteristics consistent with those of nonlinear chaotic systems. Dimensional analysis resulted in three distinct groups being identified. Results indicate that control of the fetal heart rate may be modeled as a nonlinear or chaotic system, and analytic techniques borrowed from the physical sciences are useful in exploring heart rate variability. That different groups could be distinguished among qualitatively similar heart rate tracings may lead to understanding of discrepancies between evaluation of the monitor tracing and neonatal outcome. (AM J OBSTET GYNECOL 1991;165:1425-9.)

Key words: Chaos, dimensional analysis, nonlinear, heart rate variability

Fetal heart rate (FHR) variability was first recognized as being clinically important by Hon,1 Hammacher et al.,2 and Caldeyro-Barcia et al.3 in the early 1960s, and the intervening years have seen intensive research into the underlying source of the variations.4 Numerous statistical indices have been developed for quantifying variability,5-7 and various physiologic models involving the integration of the autonomic, humoral, and central nervous systems have been proposed.4 The central tenet of this article is that the limitations of the above approaches derive from the constraint of linearity. In linear systems the measured variable (such as heart rate) is presumed to be the weighted sum of the various factors that influence that variable. In general, small perturbations of the controlling factors will give small changes in the measured variable. In contrast, in a nonlinear system no such presumption is made. A nonlinear system may give rise to chaos where long-term predictability is impossible, and small perturbations may result in radically new behavior in the measured variable.8.9 Recent work in systems ranging from the intact adult human10 to myocardial cell aggregates11 suggests that control of heart rate may be best described with nonlinear dynamics. With phase space reconstruction (plotting each R-R interval vs the R-R interval after a fixed delay), West and Goldberger10 obtained graphs (attractors) suggesting that the fluctuations in a normal heart rate are typical of a chaotic nonlinear system. They showed that as disease progresses there is a loss

170 160 140 140 150 160 170 FHT(t)

Fig. 1. Phase-space attractor from case 4. Successive values of FHR are plotted against those 10 beats later.

of this chaotic nature, ultimately leading to a stable point (no variability) immediately preceding death. In studies of periodically perturbed, spontaneously contracting aggregates of myocardial cells Guevara¹¹ has found period doubling and phase locking, features that are consistent with nonlinear dynamics. This article attempts to show that tools traditionally used in the analysis of nonlinear dynamic systems, including phase-space graphs and dimensional analysis, may be applied to FHR variability.

Material and methods

Heart rate data were obtained from scalp electrodes on 12 fetuses at term, in labor, with an FHR tracing (FHT) pattern considered reactive by the management team. All fetuses were delivered in good condition with

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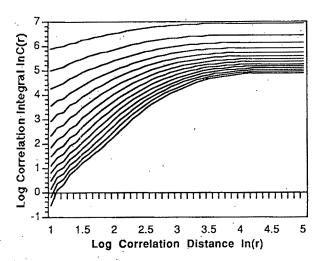


Fig. 2. Log/log plot of correlation integral versus distance for case 4. Each curve is plot of lnC(r) versus distance at embedding dimensions 2 through 15. Slope of linear region of each curve between 2 and 3.5 on ln(r) axis is correlation dimension v for corresponding embedding dimension.

a 5-minute Apgar score >8. The electrodes were attached to a Corometrics 112 cardiotocodynamometer, which strobes a voltage proportional to the R-R interval of the fetal electrocardiogram onto a port at the back of the machine. This voltage was digitized at 12 bits of precision, with a GW Instruments A/D converter with an effective precision of ± 1 beat/min. A total of 5000 heartbeat intervals for each fetus were stored on a Macintosh computer for off-line analysis.

Phase-space attractors were created by using a time-delay technique to produce a two-dimensional vector from a one-dimensional time series. FHT at time t is plotted against FHT at time t+10 beats, with a delay of 10 beats empirically chosen as giving the best resolution of detail (a representative attractor is shown in Fig. 1).

Dimensional analysis was performed by means of the method of Grassberger and Procaccia. ¹² Briefly, a point in n-dimensional phase space may be formed by n successive time-delayed values of the FHT, where the time delay k=10 beats:

[FHT (t), FHT (t + k), FHT (t + 2k), ..., FHT
$$(t + nk)$$
]

For each n-dimensional phase space, n=2 to 15 (5000 -n) points were so created; this is the process of embedding the FHT in the n-dimensional space. For example, the attractor in Fig. 1 is embedded in two dimensions (with [FHT (t), FHT (t+10)]). Within each embedding dimension, the distance (r) of each point to every other point was then calculated. The range of distances, from the smallest to the largest, was broken up into discrete intervals, and the number of times a distance fell within an interval was counted. From this

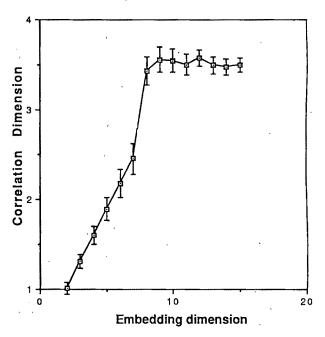


Fig. 3. Correlation dimension (\pm SE) versus embedding dimension for case 4. Plateau region beginning at embedding dimension 9 is well defined (mean, 3.519 ± 0.016).

Table I. Calculated dimensions of attractors

Case No.	Dimension	Standard error	
1	NC		
2	NC	٠,	
3	4.034	0.073	
4	3.519	0.016	
5	3.899	0.019	
6	4.358	0.055	
7	3.786	0.006	
8	3.963	0.020	
9	4.591	0.053	
10	4.865	0.036	
11	2.268	0.037	
12	1.067	0.023	

NC, Not calculable.

histogram the cumulative histogram was formed by summing the number of instances that a distance was less than or equal to the upper boundary of the interval. For example, the value of the cumulative histogram at 0.0498 is the count of the number of instances that an interpoint distance (r) was ≤ 0.0498 . This cumulative histogram is the correlation integral C(r). The logarithm of the correlation integral was then plotted against the logarithm of the distance, resulting in the sigmoid-shaped curve expected of a chaotic attractor. In the linear portion of the curve the correlation integral should scale as lnC(r) = vlnr, where v is the dimension of the attractor. The slope (v) of the linear portion of the curve was calculated with simple linear regression over the longest segment with a regression

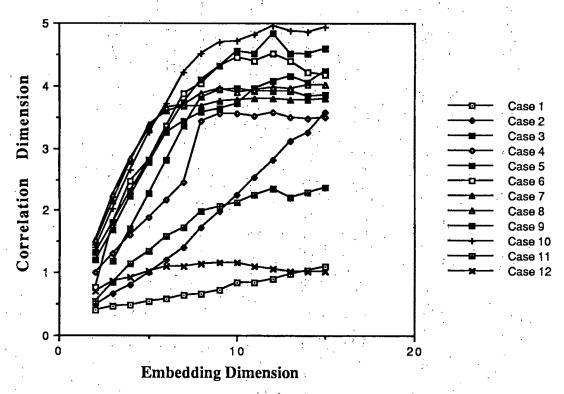


Fig. 4. Correlation dimension versus embedding dimension for 12 fetuses.

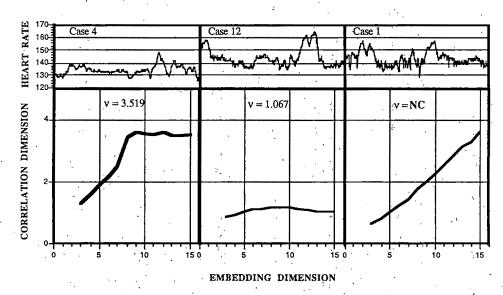


Fig. 5. Comparison of illustrative cases from three categories of dimension, v, with associated FHTs. NC, Not calculable.

coefficient $R^2 > 0.98$. This process was carried out for successively higher embedding dimensions (n = 2 to 15). Where a plateau was observed in the plot of the correlation dimension versus the embedding dimension, the value of the plateau was calculated with a weighted average technique where each value in the

plateau region is weighted by the variance of the underlying slope calculation.

Results

Random noise is uncorrelated over all characteristic distances and in phase space shows no structure; a lin-

ear system would have a very simple structure, either a point or a closed curve.¹³ However, a complicated structure is clearly evident in the attractor depicted in Fig. 1. The structure is also qualitatively similar to the attractor obtained from the heart rate of a normal adult.¹⁰

A typical plot for one fetus of the log of the correlation integral [lnC(r)] versus the logarithm of the distance [ln(r)] for embedding dimensions 2 to 15 is shown in Fig. 2. The linear portion of each curve is termed the scaling region. The slope of the scaling region for each curve was calculated and the results were plotted against the embedding dimension in Fig. 3. Noise, or a purely stochastic process, results in slopes that continue to increase without bound as the embedding dimension increases, whereas a linear system would give an integral correlation dimension. However a nonintegral plateau region is clearly seen, implying that a low-dimensional attractor is present, and the dimension of this attractor is 3.519 ± 0.016 as calculated from the weighted average of the plateau values. The results of similar plots for all 12 fetuses are shown in Fig. 4 and the resulting dimensional calculations are shown in Table I. Three distinct states are noted: a low-dimensional attractor with a correlation dimension between 3 and 5 (cases 3 through 10), a low-dimensional attractor of dimension ≈1 (cases 11 and 12), and an uncalculated dimension in two cases (a dimension could not be calculated because the correlation integral continued to increase with increasing embedding dimension [cases 1 and 2]). FHTs and correlation dimension versus embedding dimension graphs representative of the three subsets are illustrated in Fig. 5.

Comment

Previous attempts to analyze the variability present in the FHR have either concentrated on statistical descriptions of the time series⁵⁻⁷ or, using the Fourier transform, reported information on the power spectrum.4 Both of these methods of analysis rely on a linear model of FHR control. The statistical descriptions were developed without an attempt to explain the underlying influences, and although the descriptions appear reliable in quantifying short-term variability, they fail to account for long-term variability and are no better than a trained clinical observer. Spectral analysis models the control of the heart rate as the linear combination of a variety of physiologic inputs and attempts to quantify the role played by each (parasympathetic, sympathetic, thermoregulatory, etc.) by the amount of spectral power at a characteristic frequency. The underlying assumption of spectral analysis models is that heart rate variability will change as a linear function of changes in spectral power at certain frequencies.

The results presented in this article suggest the FHR is inherently nonlinear in nature and that there is in-

formation to be gained from characterizing the behavior of the dynamics of heart rate in phase space. A suggestion that this is a reasonable approach came from visual inspection of the two-dimensional plot, which is clearly not a simple limit cycle (circle) and neither is it consistent with noise (i.e., a scattergram). Linear systems are limited in the structures they can display in phase space to a simple circle (or smooth deformation of a circle, such as an ellipse), whereas chaotic systems may have rather bizarre paths (attractors) in phase space.18 One way to characterize an attractor is by quantifying the dimension,12 which can be viewed as an approximation of the number of variables needed to describe the dynamics. It is characteristic of chaotic (as opposed to stochastic) dynamics that the dimension of the attractor in phase space is less than the dimension in which it is embedded. It is not the purpose of this article to assert that the resulting number has any independent meaning in and of itself (given the limitations of noise, measurement inaccuracy, and small data sets); rather the point is that the FHR exhibits nonlinear dynamics (chaos) and is amenable to analytic techniques differing from those for the traditional linear model.

In this report the FHTs would be interpreted as reactive by conventional criteria, and each labor resulted in a vigorous newborn. However, dimensional analysis revealed several distinct subcategories, which may correspond to different physiologic states that are indistinguishable by simple inspection of the monitor tracing. Fifty percent of fetuses with an abnormal FHT (late decelerations) have no evidence of acidosis at birth14; dimensional analysis may be able to distinguish an acidotic fetus from a normal fetus in such cases. Whereas further studies will be necessary to characterize the physiologic and clinical correlates of these measures of phase-space dynamics, this study suggests that nonlinear analysis of human fetal physiologic characteristics may be a fruitful alternative to more traditional models.

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Identifying pregnant women who drink alcoholic beverages

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This randomized 2×2 study compared disclosure rates of alcohol use with two response formats (multiple choice and dichotomous) and two communication channels (oral and written) in an adult prenatal population (N = 1078). The multiple choice question improved disclosure, regardless of channel, by 40% across white, African-American, and Hispanic subgroups. (AM J OBSTET GYNECOL 1991;165:1429-30.)

Key words: Prenatal risk assessment, prenatal history taking, self-reported alcohol consumption

Obstetricians need accurate information about their patients' alcohol consumption. Many pregnant women do not report use of alcohol, and some physicians are reluctant to broach this sensitive area. Available screening tools for general patient populations are not very helpful with pregnant women and provide information only about abusive drinking or alcoholism. Therefore we tested a question to identify all pregnant drinkers of alcohol, regardless of amount of consumption.

Material and methods

Two response formats ("yes or no" and multiple choice, Table I) and two channels of questioning (oral and written) were compared, by means of a random-

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Table I. Multiple choice question

Which of the following statements best describes your use of any alcoholic beverages, including beer, wine, and hard liquor?

Would you say:

- I drink regularly now—about the same amount as before finding out I was pregnant.
- 2. I drink regularly now, but I've cut down since I found out I was pregnant.
- 3. I drink every once in a while.
- 4. I have quit drinking since finding out I was pregnant.
- 5. I wasn't drinking around the time I found out I was pregnant, and I don't currently drink.

ized, 2 × 2 factorial design. In all study groups we combined the alcohol history question with questions about use of automobile safety belts, smoking, and illicit drugs. All study subjects were English-speaking women ≥18 years old who were free of mental and sensory handicaps and who entered prenatal care at one of four multispecialty group satellite clinics between September 1988 and February 1990.

The experiment was conducted during the first visit after confirmation of pregnancy. The experimental questions were embedded in the oral and written history questions. Prenatal history information was stripped of identifiers before being given to university-based researchers, a procedure approved by the uni-

versity and clinic human subjects protection committees.

Results

Of 1206 pregnant women seen for care during the study, 1078 (89.4%) were eligible. The sample was Hispanic (14.7%), African-American (32.6%), and white, non-Hispanic (50.1%); married (82.5%); with a mean age of 28 years; with low parity (80.7% with <2 previous live births); and with few maternal or previous fetal or infant health problems (75.5% had none). The groups did not differ according to race or ethnicity, marital status, age, parity, or maternal health problems or fetal or maternal problems in previous pregnancies.

We used a logistic regression model for data analysis. The primary dependent variable was self-reported drinking, either a "yes" response to the dichotomous question or a "yes" response to any of the first three categories on the multiple choice question. The multiple choice format elicited a positive drinking response that was five percentage points higher (13.58% vs 8.47%; odds ratio 1.70, 95% confidence interval 1.15 to 2.51, p < 0.01). There were no differences among ethnic or racial groups.

Within the multiple choice question, responses indicating current drinking were as follows: cut back for pregnancy (61.6%), drink occasionally (93.0%), and drink regularly (0.01%). The channel of questioning, oral or written, did not produce significantly different levels of self-report (odds ratio 1.24, p < 0.28), and there was no interaction of format and channel.

Comment

Using a multiple choice question administered in either written or oral channels encouraged more disclosure of alcohol use by this population of pregnant women than did a dichotomous format. Although the study is limited by the absence of biologic confirmation of self-report, we believe these results reflect a true relative difference.

Why did the difference occur? For smoking, the response option that allows the woman to lessen the undesirability of smoking by saying that she has cut down in pregnancy accounted for 62% of the response among those indicating smoking. With drinking, this response was chosen infrequently. Occasional drinking was selected by 93% of the respondents who said they drank at all (vs 22% among smokers). This may correctly describe some or many of those selecting this response. It might also have been selected by binge drinkers and regular heavy drinkers who have not admitted to themselves the extent of their drinking or who are trying to make a plausible statement to decribe their drinking behavior.

Within the structure of this experiment, we cannot know, however, the absolute sensitivity of the question or which types of drinkers were captured in the increased disclosure rate. The true rate of drinking in the population may be closer to 25%. However, even moderate consumption may be of interest. Thus we recommend using the multiple choice question for screening only, ideally not as the exclusive method. The most useful follow-up questions may be direct probing of amount and alcohol-related problems. All pregnant women should be screened. Future research is needed on assessment techniques in pregnancy; more important, research is needed to develop interventions.

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Human papillomavirus infection in women with multicentric squamous cell neoplasia

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Tissues from 32 women with multicentric squamous cell neoplasia of the anogenital region (72 anatomically distinct lesions at the cervix, vagina, vulva, perineum, or anus) were tested for the presence of human papillomavirus with the polymerase chain reaction or in situ hybridization. All the women had invasive carcinomas or grade 3 intraepithelial neoplasia lesions at a minimum of one site and one or two squamous cell lesions at another site(s). Human papillomavirus was detected in all of the multicentric lesions in 87.5% (28/32) of the women and in at least one lesion in 12.5% (4/32). In the 28 women with detectable human papillomavirus at all sites, 61% (17/28) had the same virus type(s) at all sites (types 6, 16, 6 and 16, 33) and 25% (7/28) had 6 or 16 at one site and both viruses at the other site(s). Four women (15%) had different virus patterns in the separate lesions. (AM J OBSTET GYNECOL 1991;165: 1431-7.)

Key words: Human papillomavirus, multicentric squamous cell neoplasia, polymerase chain reaction

Numerous studies have reported that infection with human papillomavirus (HPV) is associated with squamous cell neoplasia of the lower female genital tract. The strongest evidence implicating HPV infection in the pathogenesis of genital tract malignancies comes from investigations of human tissues with molecular hybridization techniques. Many such studies have demonstrated that most squamous cell intraepithelial neoplasias and invasive carcinomas of the cervix and vulva contain HPV deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). 1-3 Of the 60 HPV genotypes that have been identified by molecular analyses of human tissues, 22 have been found in anogenital lesions in both women and men.4 The spectrum of clinical conditions associated with HPV infection ranges from occult infections, where the viral DNA is present without cytologic or histologic evidence of infection, through condylomas to precancers and invasive carcinomas.2 HPV type 16 is the most common virus found in cervical squamous cell carcinomas and in high-grade intraepithelial lesions. 1-8 In contrast, HPV type 6 is found in the majority of vulvar, penile, and anal condylomas and is infrequently detected in high-grade intraepithelial lesions or invasive carcinomas.1.2 However, some studies have shown HPV type 6 to be present in vulvar squamous cell cancers.3 Other virus types (HPV types 18, 31, 33, 35, and 39) also are found in genital lesions but are less common than HPV types 16 and 6.1

The presence of HPV nucleic acids in squamous cell carcinomas of the cervix, vulva, vagina, and anus is consistent with a role for these viruses as etiologic agents in this morphologic type of anogenital cancer. The epidemiologic basis for considering cervical cancer as a sexually transmitted disease is well established.5 Recent epidemiologic studies have demonstrated that vulvar,6 vaginal,7 and anal8,9 cancers also have some similar risk factors with sexually transmitted diseases (number of sex partners, age at first intercourse, sexual practices). Although the proportion of squamous cell lesions that contain HPV is similar among these anatomic sites, the corresponding incidence of cancer varies markedly. In the United States the age-adjusted incidence of cervical cancer (8.0/100,000 in 1981 through 1985) is five to 10 times higher than that for carcinoma of the vulva (1.5), vagina (0.7), or anus (0.9) during the same time period.10 Also, the behavioral risk factors (number of sex partners, age at first intercourse, smoking history) for development of squamous cell carcinoma at each of these sites are somewhat different.5-9

In this study we have investigated this apparent differential pathogenicity of HPV infection in the female genital tract. We chose a study group of women with multicentric squamous cell neoplasia to determine if

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Table I. Summary of 72 lesions from 32 women with multicentric neoplasia by site, histopathologic type, and HPV result

			Primary lesion*			Second lesion			Third lesion	
Subject No.	Age (yr)	Site	Pathologic condition	HPV†	Site	Pathologic condition	HPV	Site	Pathologic condition	HPV
1	66	Cervix	M-Inv	16	Vagina	Inv	16			_
2	60	Cervix	Inv	35	Vagina	Inv	Neg		*****	_
3	32	Cervix	Inv	16	Vagina	Inv	16			_
4	45	Cervix	M-Inv	6, 16	Vagina	Grade 3 IN	16		,	_
5	36	Cervix	Grade 3 IN	6, 16	Vulva	Grade 3 IN	6, 16	Vagina	Grade 1 IN	6, 16
6	26	Cervix	Grade 3 IN	16	Vulva	Inv	16	Anus	Condyloma	33
7	23	Cervix	Grade 3 IN	(16)	Vulva	Condyloma	6, 16	Anus	Condyloma	6, 16
8	61	Vagina	Grade 3 IN	16	Cervix	Grade 3 IN	16		· :	_
9	62	Vagina	Grade IN	16	Vulva	Grade 3 IN	16		*****	_
10	62	Vagina	Grade 3 IN	16	Vulva	Condyloma	6			_
11	52	Vulva	M-Inv	16	Cervix	M-Inv	Neg			
12	40	Vulva	Grade 3 IN	16	Cervix	Grade 3 IN	UK		*****	_
13	34	Vulva	Grade 3	6	Cervix	Grade 3	6			_
			IN/condyloma			IN/condyloma				
14	['] 41	Vulva	Grade 3 IN	16	Cervix	Grade 3 IN	16			_
15	69	Vulva	Grade 3 IN	Neg	Cervix	Grade 3 IN	Neg	Vagina	Grade 3 IN	35
16	28	Vulva	Grade 3 IN	(16)	Cervix	Grade 2 IN	(16)			_
17	36	Vulva	Grade 3 IN	16	Cervix	Grade 1 IN	· 16			
18	59	Vulva	Grade 3 IN	33	Vagina	Grade 3 IN	6			_
19	66	Vulva	Grade 3 IN	6, 16	Vagina	Grade 3 IN	6, 16			_
20	60	Vulva	Grade 3 IN	UK	Vagina	Grade 3 IN	ÚK			_
. 21	25	Vulva	Grade 3 IN	33	Vagina	Condyloma	33		-	_
22	36	Vulva	Grade 3 IN	6, 16	Vagina	Grade 2 IN	6, 16			_
23	. 40	Vulva	Grade 3 IN	(16)	Vagina	Grade 1 IN	Neg			
24	35	Vulva	Grade 3 IN	6, 16	Vagina	Grade 1 IN	6, 16			_
25	55	Vulva	Grade 3 IN	(16)	Vagina	Grade 1 IN	(16)			
26	42	Vulva	Grade 3 IN	16	Vagina	Grade 3 IN	16	Anus	Grade 3 IN	16
27	41	Vulva	Grade 3 IN	16	Vagina	Grade 3 IN	6, 16	Anus	Grade 3	6, 16
28	42	Vulva	Grade 3 IN	6, 16	Vagina	Condyloma	6	Anus	IN/condyloma Condyloma	6
29	49	Vulva	Grade 3 IN	16	Perineum	Grade 3 IN	6, 16			_
30	36	Vulva	Grade 3 IN	6, 16	Anus	Grade 3 IN	6, 16	,		_
31	40	Vulva	Grade 3 IN	16	Anus	Grade 3 IN/condyloma	6, 16	_		_
32	32	Vulva	Grade 3 IN	- 16	Anus	Grade 3 IN	6, 16	Cervix	Grade 1 IN	6

M-Inv, Microinvasive squamous cell carcinoma; Inv, invasive squamous cell carcinoma; IN, intraepithelial neoplasia.

†HPV types in parentheses were determined from in situ hybridization analyses; Neg, negative; UK, unidentified type (not 6, 11, 16, 18, 31, 33, 35, 39, or 42).

infection with viruses of different oncogenic potentials (i.e., HPV type 6 or 16) was associated with individual squamous cell lesions at different epithelial surfaces in the female genital tract. Our objectives were (1) to determine if the same virus type was present in carcinoma in situ or invasive squamous cell carcinomas at different anogenital sites in the same individual and (2) to determine if different virus types were present in lesions of different malignant potentials at various epithelial surfaces in the same individual.

Material and methods

Subject tissues. Women were chosen for this study by surveys of pathology reports of individuals enrolled in case-control studies of cervical, vaginal, and vulvar cancer being conducted in western Washington State (principal investigator, Janet R. Daling, PhD). Women were eligible for inclusion if they had been diagnosed

with either invasive squamous cell cancer or grade 3 intraepithelial neoplasia at one site (cervix, vulva, or vagina) and had concurrent invasive cancer, grade 3 intraepithelial neoplasia, grade 1 or 2 intraepithelial neoplasia, or HPV-related morphologic changes in at least one other anogenital site (cervix, vulva, vagina, perineum, or anus).

The study group included 22 women with invasive cancer or grade 3 intraepithelial neoplasia at more than one site and 10 women with grade 3 intraepithelial neoplasia at one site and with lesions at other sites graded 1 or 2 intraepithelial neoplasia or HPV-related morphologic changes. These 32 women had lesions at 72 distinct anatomic sites (Table I) and were chosen for this study because paraffin-embedded fixed tissues were available from all of the lesions.

Histologic criteria. Anogenital squamous cell lesions were classified in three groups for this study: (1) in-

^{*}Primary lesion defined as that tumor for which patient was identified for case-control studies.

vasive or microinvasive squamous cell carcinoma, (2) cervical, vulvar, vaginal, or anal grade 3 intraepithelial neoplasia, and (3) other intraepithelial neoplasia lesions or morphologic changes indicative of HPV infection (condyloma or koilocytotic atypia at the cervix). Histopathologic diagnoses were determined from pathol-

Detection of HPV nucleic acids. The polymerase chain reaction was used to detect HPV DNA in 66 of the 72 lesions. Many of the lesions had already been analyzed by in situ hybridization before the development of the polymerase chain reaction method, and all of the available paraffin-embedded material had been used for in situ hybridization tests. Consequently, for this study adequate amounts of remaining tissue for polymerase chain reaction tests were not available for six of the 72 lesions, and in situ hybridization with asymmetric RNA probes was the only means for detection of HPV nucleic acids in these six lesions.

Polymerase chain reaction. Paraffin-embedded tissues were prepared for polymerase chain reaction analyses as described by Wright and Manos,11 and the polymerase chain reaction was performed on cellular DNA extracted from single 6 µm sections. Initially, tissue DNAs were amplified with oligonucleotide primers specific for the E6/E7 region of the HPV type 6 or 16 genomes.12 All positive results obtained with primers from the E6/E7 region were confirmed by amplification of a second region of the HPV type 6 or 16 genome; these were the El open reading frame (ORF) for HPV type 6 and the L1 ORF for HPV type 16. For the HPV type 6 E1 ORF, oligomer primers were GTTGCTGTG-GATGTGACAGCAACGT (nucleotides 699 to 723) and CCTGACCATCTCCCCCCATTTTCCGG (nucleotides 1321 to 1297). Both to confirm HPV 16 positive results and also to reanalyze tissue DNAs that initially tested negative by polymerase chain reaction with HPV type 6 or 16 E6/E7 primers, a set of degenerate consensus primers were used for the amplification reactions.18 These primers amplify a region of sequences common to at least 25 distinct genital HPV types,18 and, after RsaI digestion of amplification products, HPV types can be distinguished by the pattern of restriction enzyme fragments.13, 14

To assure maximum sensitivity and specificity of the polymerase chain reaction, all reaction products were electrophoresed through 4.0% agarose gels, transferred to nitrocellulose by Southern's method,15 and hybridized with type-specific or consensus probes derived from viral DNA sequences internal to the primer pairs. This approach for analysis of HPV genomes has been previously described by our group. 12, 14 For the HPV type 6 E1 ORF the probe sequence was AT-TATGCGACTGTGCAGGACCTAAAACGAAAGT-ATTTAGG (nucleotides 1055 through 1093); the probe sequences for the HPV types 6 and 16 E6/E7 ORF and the L1 consensus sequence have been described elsewhere.12,14 The amplification reactions allow consistent and reproducible detection of 0.1 pg of plasmid viral DNA after hybridization, representing about 10³ copies of the HPV genome.

In situ hybridization. In situ hybridization used asymmetric RNA probes from the E6/E7 region of the HPV types 6 or 16 genomes essentially as described by Stoler and Broker.16 Briefly, sections were dewaxed, digested with proteinase K (15 μg/ml), and acetylated (0.25% acetic anhydride in 0.1 mol/L triethanolamine, pH 8.0). Sections were hybridized overnight at 49° C, in stringent conditions, with 1.0 to 5.0×10^7 disintegrations/min of tritium-labeled HPV type 6 or 16 RNA. Antisense RNA probes (complementary to the messenger RNA made in vivo) were generated from HPV type 6 or 16 E6/E7 ORFs cloned into the transcription vector pSP6-T7-19 (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Hybridized sections were washed, dehydrated, coated with autoradiographic emulsion (Kodak NTB-2), and exposed for 4 weeks. Tissues were stained with hematoxylin for microscopic evaluation.

Results

Anatomic distribution of lesions. All 32 women in this study had two or three squamous cell lesions at various sites throughout the anogenital region; the site and histopathologic type of these neoplasias are detailed in Table I.

Lesions at the vulva and vagina (n = 10; age range 26 to 66; median, 57) were the most common in this study. Four of these women had grade 3 vulvar and vaginal intraepithelial neoplasia, five had grade 3 vulvar intraepithelial neoplasia and less severe vaginal lesions, and one had grade 3 vaginal intraepithelial neoplasia and a vulvar condyloma. Six women had lesions at the cervix and vulva (age range, 28 to 52; median, 38). Four of these women had invasive cancer or grade 3 intraepithelial neoplasia at both sites, and two had grade 3 vulvar intraepithelial neoplasia and less severe cervical lesions. Five women had lesions at the cervix and vagina (age range, 32 to 66; median, 60); all had either invasive cancer or grade 3 intraepithelial neoplasia at both sites. Two women had grade 3 intraepithelial neoplasia lesions at the vulva and anus (ages 36 and 40), and one women had grade 3 vulvar intraepithelial neoplasia and a distinct grade 3 intraepithelial neoplasia lesion on the perineum (age

Eight women had lesions at three sites. Three individuals had involvement at the cervix, vulva, and anus (ages 23, 26, 32); three had lesions at the vulva, vagina, and anus (ages 28, 41, 42); and two had lesions at the cervix, vagina, and vulva (ages 36, 69).

Although the numbers of women in each group outlined above are small, it appears that women with vag-

Table II. Detection of HPV DNA in 72 lesions from 32 women with multicentric squamous neoplasia

Anatomic site and			HPV type in p	ositive spe	cimens		HPV	7	otal
histopathologic type of lesion	6	16	6 and 16	33	35	Unknown	DNA negative	No.	%
Cervix	-								
Grade 1 or 2 CIN*	1	2	0	0	0	0	0	3	100
Grade 3 CIN	1	3	2	0	0	1	1	8	87.5
Invasive cancer	0	2	1	0	1	0	1	5	80
Vulva .									
Condyloma	1	0	1 .	0	0	0	0	. 2	100
Grade 3 VIN	1	12	6	2	0 .	1	1	23	96
Invasive cancer	0	2	0	0	0	0	0	2	100
Vagina									
Čondyloma	1	1	3	1	0	0	1	7	86
Grade 1 or 2 VAIN†			•						
Grade 3 VAIN	1	5	2	0	1 '	. 1	0	10	100
Invasive cancer	0	2	0	0	0	0	1	3	67
Anus									
Condyloma	. 1	0	1	1	0	0	0	3	100
Grade 3 AIN	0 .	1	4	0	0	0	0	5	100
Perineum					. !				
Grade 3 IN	0	0	1	0	0	0	0	1	100

CIN, Cervical intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia; VAIN, vaginal intraepithelial neoplasia; AIN, anal intraepithelial neoplasia.

inal and either cervical or vulvar lesions were older than women with lesions involving the vulva and either the cervix or anus.

Detection of HPV nucleic acids. A total of 276 tissue blocks comprising 72 anatomically distinct lesions from 32 women were analyzed by polymerase chain reaction or in situ hybridization. Sixty-seven of the 72 lesions (93%) contained HPV DNA or RNA of types 6, 16, 33, 35, or unidentified HPV types (Tables I and II). HPV nucleic acids were more frequently detected in anal or perineal (100%; n = 9) and vulvar (96%; 26/27) lesions than in vaginal (90%; 18/20) or cervical (88%; 14/16) lesions (Table II).

When the histopathologic type of all lesions is considered, eight of nine (89%) condylomas contained detectable HPV DNA, six of six lesions (100%) graded 1 or 2 intraepithelial neoplasia were positive, 45 of 47 (96%) grade 3 intraepithelial neoplasia lesions were positive, and eight of 10 (80%) invasive cancers were positive (Table II). The negative condyloma was a flat lesion in the vagina of a woman with a grade 3 vaginal intraepithelial neoplasia lesion that contained HPV type 16 DNA. The two negative grade 3 intraepithelial neoplasia lesions were at the vulva and cervix of a woman who also had grade 3 vaginal intraepithelial neoplasia that contained HPV type 35 DNA. Of the two negative invasive cancers, one was a second primary cancer of the vagina in a woman with invasive cancer of the cervix that contained HPV type 35 DNA, and the second was an invasive cervical cancer in a woman with invasive vulvar cancer that contained HPV type 16 DNA. All of the HPV-negative tissues had been tested with the polymerase chain reaction; the less sensitive in situ hybridization method was not responsible for these negative results. Also, these tissues were tested with primer pairs for the human β -globin gene. ^{18, 17} All the HPV-negative lesions gave positive results with the β -globin primers (data not shown), demonstrating the adequacy of the DNA preparations for polymerase chain reaction analyses.

Distribution of HPV types by anatomic location of lesions. In the 67 lesions that contained detectable HPV nucleic acids, HPV type 6 or 16 was found in tissues from all anatomic sites, whereas HPV type 33 was seen only at the vulva, vagina, and anus, and HPV type 35 only at the cervix and vagina (Table II). HPV type 16 infection was more common at the cervix, vulva, and vagina than at the anus, whereas mixed infection with HPV types 6 and 16 was most common at the anus.

At all sites, as the degree of neoplasia increased, so did the proportion of HPV type 16-positive lesions (Table III). Double infections with HPV types 6 and 16 were approximately twice as common in noninvasive lesions; only one invasive cancer, at the cervix, contained both types 6 and 16 (Table II). HPV type 16 was the most common virus type found in invasive cancers or grade 3 intraepithelial neoplasia lesions of the cervix (5/11), vulva (14/24), or vagina (7/12). However, women with anal intraepithelial neoplasia were more likely to have a dual infection with HPV types 6 and 16 (4/5) than an infection with HPV type 16 alone (1/5) (Table II).

Virus patterns in multicentric lesions. HPV nucleic acids were detected in all of the anatomically distinct

^{*}Includes one grade 2 cervical intraepithelial neoplasia with HPV type 16.

[†]Includes one grade 2 vaginal intraepithelial neoplasia with HPV types 6 and 16.

	HPV type in positive specimens												
Histopathologic type of lesion	6		16		6 and 16		33		35		Unknown		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	Total (No.)
Condyloma, grade 1 or 2 IN	4	29	3	21	5	36	2	14	0	0	0	0	14
Grade 3 IN Invasive cancer	3 0	7 0	21 6	47 75	15 1	33 12.5	2 0	4 0	I 1	$\begin{array}{c} 2 \\ 12.5 \end{array}$	3 0	7 0	45 8

Table III. HPV type-specific disease association in virus-positive multicentric lesions

IN, Intraepithelial neoplasia.

lesions in 87.5% (28/32) of the women in this study. Four women (subjects 2, 11, 15, and 23) had detectable HPV at only one site; three of these women had lesions at two sites and one had lesions at three sites (Table I).

The proportion of women with HPV DNA or RNA at all sites was not dependent on whether lesions were present at two or three sites (87.5% positive in both groups; 21/24 and 7/8, respectively). In women with lesions at two sites, the proportion of individuals with HPV at both sites did not depend on the degree of neoplasia. For women with grade 3 intraepithelial neoplasia at two sites, 87.5% (14/16) were positive at both sites. For women with grade 3 intraepithelial neoplasia at one site and less severe neoplasia at the other involved site, 87.5% (7/8) were positive at all three sites. In women with lesions at three sites, HPV was found in five of six individuals (84%) with grade 3 intraepithelial neoplasia at all sites and in both women with one grade 3 intraepithelial neoplasia lesion and less severe neoplasia at the other two sites.

In the 28 women with detectable HPV at all sites, 61% (17/28) had the same virus type(s) at all sites and 25% (7/28) had HPV type 6 or 16 at one site and both viruses at the other site(s) (Table I). Four women (14%) had different virus patterns in the separate lesions (subjects 6, 10, 12, and 18; Table I).

Occurrence of double HPV infections. Thirteen of the 32 women (41%) with multicentric neoplasia and 22 of the 72 lesions (31%) showed evidence of infection with HPV types 6 and 16. Six of the 32 women (19%) were infected with HPV type 6 and 16 at more than one site, and seven of 32 (22%) were infected with both types at only one site.

The total proportion of women with both HPV type 6 and 16 infections was identical in those individuals with grade 3 intraepithelial neoplasia at multiple sites (41%, 9/22) and those with grade 3 intraepithelial neoplasia at one site and lesions less than grade 3 intraepithelial neoplasia at other sites (40%, 4/10). In the first group of women, four of 22 (18%) individuals had double infections at more than one site, and five of 22 (23%) had double infections at only one site. In the second group, two of 10 women (20%) had double infections at more than one site and the same proportion had double infections at only one site.

Comment

In this study of women with multicentric squamous cell neoplasia of the lower genital tract, all of the 32 women had molecular evidence of HPV infection at at least one site. Five lesions from four individuals lacked detectable HPV nucleic acids; in each instance either an invasive cancer or grade 3 intraepithelial neoplasia at another site was HPV positive. Failure to detect HPV DNA in genital squamous cell carcinomas by conventional hybridization techniques (i.e., Southern, dot blot, in situ) may reflect a sampling error (missing the lesion), the presence of small amounts of virus that would not be detected by the methods used, the presence of a virus type unrelated to the probes, or the absence of HPV in the tumor. In this study the lack of HPV in these lesions is unlikely to represent a sampling error as sections from the entire tumor were analyzed in all cases. The sensitivity of the polymerase chain reaction is such that viral DNA should be detectable if present; however, the late consensus primers may not detect all HPV types that can infect the genital tract. A more convincing explanation for these negative findings is that random nicks introduced into the viral DNA after storage of paraffin tissues removed the oligonucleotide primer binding sites. Other investigators have demonstrated random loss of amplification ability of the human β-globin gene, which increases with the age of the paraffin-embedded tissue.¹⁷ Alternately, the HPVnegative lesions may not contain any viral nucleic acids.

Lower rates of HPV positivity than what was found in this study have been reported in previous studies of women with multicentric squamous cell genital lesions. Pilotti et al.18 used in situ hybridization to study tissues from women with multicentric disease and found that 73% (19/26) were positive for HPV DNA. These women were selected by clinical observation of vulvar wart-like lesions and simultaneous lesions at other sites, and approximately one half of the women had grade 3 vulvar intraepithelial neoplasia and the other 1436 Beckmann et al.

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half had condylomatous vulvar lesions. Bergeron et al. 19 found HPV DNA by Southern hybridization in 87.5% (21/24) of women with multiple condylomatous lesions of the anogenital region. Kulski et al.20 found evidence of HPV infection in 85% (109/128) of women with cervical abnormalities who also had vulvar tissues collected for analysis by filter in situ hybridization. The 100% patient positivity rate in this study is, most likely, a result of the use of polymerase chain reaction, a test that is many times more sensitive than other detection methods. However, when considering each lesion separately, we found lower rates of HPV detection in the cervices of affected individuals than were seen at other sites in the genital tract. These results are similar to those of Reid et al.,21 who found detection rates, in cervical and vulvar biopsy specimens that showed koilocytotic atypia or neoplasia, of 86% and 93%, respectively. Conceivably, a wider variety of HPV types infect the cervix than the vulva, and these types infecting the cervix are not detectable under our experimental conditions.

The relative frequencies of vulvar, cervical, vaginal, or anal HPV infections occurring simultaneously, in any combination, are not known. Some studies have shown that in women with vulvar condyloma or grade 3 vulvar intraepithelial neoplasia up to 60% also have cervical HPV infections or preneoplastic lesions. 22-24 Conversely, in women diagnosed with HPV-related cervical lesions, more than one half also have vulvar lesions.20 In 1984, Okagaki25 proposed the term genital neoplasm papilloma syndrome to describe this clinical observation of synchronously or metachronously occurring multiple HPV infections or HPV-associated intraepithelial neoplasia lesions in the female genital tract. Also, epidemiologic studies have shown a significant association between the simultaneous detection or occurrence of cervical and vulvar cancers and squamous cell cancers at other anogenital sites,26,27 implicating a common etiologic agent.

The patterns of viral distribution in the multicentric lesions examined in this study suggest that the majority of these neoplasias were the result of diffuse HPV infection. Four women had different HPV types found in different areas of the genital tract; of these, two had two lesions of the same histologic grade (grade 3 vulvar intraepithelial neoplasia and grade 3 vaginal intraepithelial neoplasia or grade 3 cervical intraepithelial neoplasia), and the other two had grade 3 vaginal intraepithelial neoplasia and a vulvar condyloma, or grade 3 cervical intraepithelial neoplasia, vulvar invasive cancer, and an anal condyloma. Our results are consistent with the idea that squamous cell malignancies of the lower female genital tract share a common etiology, of which one factor is HPV infection.

Women with multicentric HPV infection, whether in-

volving benign condylomas, precancers, or invasive lesions, appear to have much higher rates of multiple infections than do groups of women with only singlesite involvement. In this study 13 of the 32 (41%) women had mixed infections throughout the lower genital tract, and 22 of the 72 (31%) individual lesions contained both HPV types 6 and 16 DNA. These results agree with other studies in which from 38% to 100% of women with multicentric lesions were infected with two or more HPV types. 18-20, 28 In contrast, studies of women with only cervical or vulvar lesions have shown low rates (<5%) of multiple HPV infections.2 This study did not analyze the exact cellular location of each virus type present in a single lesion. Reid et al.21 have shown that in individuals with multiple HPV infections the different virus types were localized in demarcated areas of differing histopathologic appearances. However, in our study most all of these lesions containing HPV types 6 and 16 DNA were of similar or identical histopathologic grades. The observation of multiple infections in women with synchronously occurring multicentric lesions suggests that this study group represents a selection of individuals in whom the effectiveness of the host response to HPV infection is inadequate.

In this study different viruses infecting differing areas of the female genital tract were ruled out as a potential explanation for the observed variation in the occurrence of squamous cell cancers at the cervix, vulvaa, vagina, and anus. HPV type 16 infection was the common thread among intraepithelial neoplasia III or invasive lesions at all sites. Interestingly, simultaneous infections with HPV types 6 and 16 were more common at the vulva and perineal and perianal skin than at the cervix, perhaps indicating a site restriction of HPV type 6 infection to cutaneous epithelium. In any event, factors other than the infecting virus type appear to be responsible for the 15-fold increase of cervical cancer over cancers at the vulva or vagina or anus. The unique properties of the cervical transformation zone²⁹ may make it more susceptible to HPV-induced oncogenesis than the native squamous epithelium is elsewhere in the lower genital tract. We are currently investigating the hypothesis that the cervix is more likely to undergo malignant changes after HPV infection than is the vulva, vagina, or anus because of site-specific variations in the expression of the HPV transforming genes E6 and E7.

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Human T-cell lymphotropic virus type I infection and pregnancy: A case-control study and 12-month follow-up of 135 women and their infants

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Human T-cell lymphotropic virus type I (HTLV-I) infection is common in Gabon, but its influence on pregnancy is unknown. A single case of acute T-cell leukemia in a pregnant woman has been reported in the literature, but, as far as we know, we present the first case-control study analyzing the relationship between HTLV-I seropositivity and the course and outcome of pregnancy. The study concerned 45 HTLV-I seropositive pregnant women matched with 90 seronegative pregnant women. None has clinical features of HTLV-I infection during pregnancy or during the year after delivery. HTLV-I seropositivity did not significantly affect the course or outcome of pregnancy. After losing maternal antibodies to HTLV-I, none of the infants had seroconversion to HTLV-I 1 year after birth. Filaria infection was correlated with HTLV-I seropositivity, but confounding factors may account for this observation. (AM J OBSTET GYNECOL 1991;165:1438-43.)

Key words: Africa, controlled study, HTLV-I, pregnancy, transmission, filaria infection

Human T-cell lymphotropic virus type I (HTLV-I) is a retrovirus that has been associated with two clinical entities, adult T-cell leukemia-lymphoma¹ and a neuromyelopathy (TSPP).² HTLV-I is mainly endemic in southern Japan, Equatorial Africa, and the Caribbean islands. The prevalence of antibodies is higher in women than in men of comparable age. The virus can be transmitted through sexual contact, intravenous drug abuse, breast milk, and blood transfusions.³ On the basis of seroepidemiologic studies of Japanese populations in endemic districts, >98% of seropositive subjects show no evidence of disease, and the cumulative lifetime risk of adult T-cell leukemia-lymphoma in those infected before age 20 is estimated at 4%.⁴

Recently, serologic studies have shown that HTLV-I is not restricted to endemic areas. In New York City and New Orleans, 24% of intravenous drug abusers have antibodies to HTLV-I.5 Among blood donors in the United States, HTLV-I seroprevalence (0.04%) is three times higher than the human immunodeficiency

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virus seroprevalence.⁶ In Paris, the seroprevalence rate is 0.6% among pregnant women.⁷ Although the effect of another retrovirus, human immunodeficiency virus, has been widely studied in pregnant women, little is known concerning HTLV-I and pregnancy. To our knowledge, only Ohba et al.⁸ have reported a case of HTLV-I—associated adult T-cell leukemia-lymphoma in a young pregnant Japanese woman.

To determine the influence of HTLV-I infection on the course and outcome of pregnancy, we conducted a case-control study of pregnant women living in an area of high HTLV-I seroprevalence and monitored the mothers and children for 1 year after delivery. The study was performed in Gabon, western Equatorial Africa, where we have previously found HTLV-I seroprevalence rates ranging from 5.0% in urban areas to 10.5% in rural areas among the general adult population.⁹

Patients and methods

Patients. The study was carried out at the general hospital in Franceville, Gabon, where most pregnant women in the Haut-ogoue province are monitored and give birth. The southern province of Gabon is mainly a rural area with 170,000 inhabitants and the capital, Franceville, counts 30,000 inhabitants. It is a transition zone between equatorial rain forest and savannah. Two main ethnic groups are represented, one originating from the savannah (group K), the other from the forest (group B). These two groups are equally represented in Franceville but live in different parts of the town,

where environmental factors, in particular, exposure to insect vectors are similar.

In Franceville the overall HTLV-I seroprevalence rate among pregnant women is estimated at 10.5%.9 In other studies we have shown that HTLV-I seroprevalence rates increase with age and that the rate is four times higher among people from the forest than among people from the savannah.9 Seroprevalence rates are similar in people of the same ethnic group living in different parts of the town but different between people of different ethnic groups.9

Between 1986 and 1988 we screened pregnant women attending the maternity unit of Franceville, after obtaining informed oral consent from them. To optimize follow-up we studied only women living in Franceville. Each seropositive pregnant woman was matched for age and ethnic group with two seronegative women attending the unit for the first time on the same day as or the day after the seropositive women. The women selected lived in Franceville and only women of the lower socioeconomic class come to the general hospital, therefore the cases and controls were probably representative of a relatively homogeneous population. The clinical examination was performed by a physician, once during the pregnancy, at delivery, and once a year later.

The following parameters were studied: (1) obstetric history, assessed in terms of gravidity and parity and the number of living and dead infants; (2) sexual activity of these women and their risk of exposure to sexually transmitted disease, assessed in terms of the cumulative number of sexual partners, contraception, history of sexually transmitted disease, active syphilis, genital erosions, and abortions¹⁰; (3) clinical workup for adult T-cell leukemia-lymphoma, including investigations of lymphadenopathy, hepatosplenomegaly above type II, and skin lesions such as erythroderma and nodule formation, and clinical tropical spastic paraparesis including investigation of spastic gait or frantic paraplegia, low back pain, spasticity and hyperreflexia of the legs and arms, extensor plantar responses, spastic bladder, and severe constipation; (4) course of pregnancy, assessed in terms of four main complications (malaria, latent syphilis serologic results, pregnancyinduced hypertension, preterm delivery) that are not logically related to viral infection but are well-known causes of maternal and fetal morbidity; and (5) outcome of pregnancy, assessed in terms of gestational age and mode of delivery. Unfortunately, women in Gabon come to the hospital in the second part of labor and it is impossible to determine the time of membrane rupture.

The infants were examined at birth, at 6 months, and at 1 year. The Apgar score at 1 minute, fetal weight, color of amniotic fluid and gross placental aspect were

used to assess fetal outcome; no other signs of fetal distress could be investigated. Blood tests for malaria and filaria, serologic tests for HTLV-I, differential blood cell counts, and cytologic examinations were routinely performed during the first visit of the pregnant women. At delivery and 1 year later, a blood sample for HTLV-I serologic testing was obtained from the women. Blood for HTLV-I serologic testing was also taken from the infants at birth, at 6 months, and at 1 year.

Laboratory and statistical methods

HTLV-I serology. Sera were tested for immunoglobulin G antibodies to HTLV-I by means of enzymelinked immunosorbent assay (ELISA) (Dupont de Nemours, Wilmington, Del.); ELISA-positive sera were retested by means of Western blot test for confirmation (Dupont de Nemours). A Western blot assay was considered positive when it showed antibodies to at least two gene products (p 19 or p24 for gag antigens and gp46 for the envelope antigen).11 A Western blot showing antibodies to only gag antigens was considered indeterminate, and such women were excluded from the study, as were those with positive ELISA and negative Western blot. Thus 11 pregnant women found to be ELISA positive and Western blot indeterminate for HTLV-I and two women who were ELISA positive and Western blot negative for HTLV-I were excluded from the study.

Syphilis serology. Sera were screened with a semiquantitative method (VDRL, Diagnostics Pasteur, Marnes la Coquette, France); positive sera were confirmed by means of *Treponema pallidum* hemagglutination assay (Behring Diagnostics). A test was considered positive if the titer was ≥1/80.

Blood parasites. Malaria (Plasmodium falciparum) and filaria, both endemic in Gabon (Loa-loa, Dipetalonema perstans), were diagnosed by thick-staining of blood smears in Giemsa.

Significance tests were based on the Student t test and χ^2 analysis with or without Yates' correction, depending on whether the expected values were >5 or between 3 and 5, for the likelihood ratio statistic and 95% confidence intervals estimates and normal approximation.

Results

Among the 48 seropositive pregnant women selected, 45 were seen at delivery and 40 1 year after delivery. Among the controls, 90 were seen at delivery and 77 1 year later. Among the cases, 38 of 45 were from ethnic group K, five of 45 from group B, and two of 45 from other ethnic groups; this distribution was similar to the ethnic distribution seen in the control group (Table I). All cases and controls were living in the Franceville area; in wooden houses (only the upper class have brick

Table I. Demographic obstetric and sexual history

	$\begin{array}{c} Cases \\ (N = 45) \end{array}$	$\begin{array}{c} Controls\\ (N=90) \end{array}$	p Value
Ethnic group			
K	38 (84.4%)	76 (84.4%)	NS
В	5 (11.1%)	10 (11.1%)	NS
Other	2 (4.5%)	4 (4.5%)	NS
Age (yr)	25.4 ± 5.5	25.2 ± 6.2	NS
Gravidity	5.1 ± 3.1	4.7 ± 2.7	, NS
Parity	3.4 ± 1	3.3 ± 2.1	NS
Abortions per woman	1.5 ± 1	1.37 ± 0.8	NS
Living children	3 ± 2.5	2.9 ± 2.3	NS
Dead children	0.4 ± 0.7	0.4 ± 0.8	NS
Sexual history		•	
Contraception	0	0	NS
Cumulative No. of partners	12 ± 15.5	13.8 ± 17.2	NS
Past sexually transmitted disease	32 (712)	63 (702)	NS

NS, Not significant.

Table II. Hematologic profile of cases and controls (midtrimester pregnancy and 1 year after delivery)

		Cases (N	I = 40)*			Controls (N = 77)*			
	Midterm			after ivery	Midterm		1 yr after delivery			
,	No.	%	No.	%	No.	%	No.	%	p Value	
Hemoglobin										
electrophoresis				H.O.			20	0.0	***	
Hb A/A	31	78	31	78	69	89	69	89	NS	
Hb A/S	9	22	9	22	8	11	8	11	NS	
Hemoglobin (gm/100 ml)	10.6 =	± 1.5	10.1 :	± 2.1	10.6	± 1.3	10.4	± 2.5	NS	
Neutrophils	4390 =	± 2300	3700 :	± 1750	4000 =	± 1500	3450 :	± 1280	NS	
Eosinophils	590 =	± 520	530 :	± 480	470 =	± 387	510 :	± 410	NS	
Lymphocytes	2650 =	± 648	2170	± 540	2750 =	± 345	1820 :	± 430	NS	
Rosette-like lym- phocyte nuclei	. ()		0	()	(0 .	, NS	
Monocytes	65 =	± 126	45 :	± 109	48 =	± 87	43 :	± 75	NS	

NS, Not significant.

*Peripheral blood cell count could be obtained both at midtrimester and 1 year after delivery for 40 cases and 77 controls.

houses). As shown in Table I, no difference between the cases and controls was observed regarding age or sexual and obstetric history. In spite of the high proportion with a past history of sexually transmitted disease, none of the cases or controls showed genital ulcerations or neurologic diseases throughout the study period.

No clinical manifestations of HTLV-I infection were found during pregnancy or 1 year after delivery. The hematologic profile was similar in the two groups at the first visit and 1 year after delivery (Table II). No HTLV-I seroconversion occurred in the control group and the Western blot pattern of all the subjects remained unchanged throughout the study.

The course and outcome of pregnancy and the fetal outcome are shown in Table III. The case group showed only slightly higher rates of preterm delivery and complicated pregnancy when compared with those of controls, but these differences were not significant

and the mean duration of pregnancy was the same in both groups.

The systematic search for parasites in the blood showed no difference between cases and controls with regard to P. falciparum; in contrast, the incidence of filaria parasites was significantly higher in the case group (46%) than in the controls (25%) (p, 0.02; χ^2 , 6.51) (Table IV). Similar results were found in ethnic groups K and B (Table V). Pregnancy outcome was not affected by parasitemia or ethnic distributions. Blood parasites were not sought in the adults 1 year after delivery.

Forty-four children of the seropositive women and 84 children of the control mothers were examined at 6 months and at 1 year of life and were clinically healthy. All the women breast-fed for ≥3 months, with the exception of one woman in the control group who weaned her baby after 3 weeks. The HTLV-I serologic status of the infants born to HTLV-I-seropositive

Table III. Course and outcome of pregnancy and fetal outcome

•	Cases	(N=45)	Controls	(N=90)	
	No.	%	No.	%	p Value
Uncomplicated pregnancy	29	64.4	70	78	NS
Malaria	6	13.3	10	9	NS
Hypertension	2	4.6	6	6.6	NS
Syphilis	3	6.6	3	2.7	NS
Preterm delivery	5	11	3	2.7	NS
Duration of pregnancy (wk)	37.1	\pm 2.3	37.4	± 1.5	NS
Spontaneous delivery	38	88	84	95	NS
Forceps delivery	2	4	2	2	NS
Cesarean section	4	8	3	3	NS
Apgar score at 1 min					
>7	39	87	76	85	NS
3-7	5	11	12	13	NS
<3	1	2	2	2	NS
Stillbirth	1	2	2	2	NS
Meconium-stained amniotic fluid	9	20	24	27	NS
Birth weight (gm)	3030	± 480	3100	± 400	NS
Placental weight (gm)	550	± 85	560	± 10	NS

NS, Not significant.

Table IV. Parasitic blood examination of cases and controls

	Cases (N	N = 40	Controls	(N = 77)	
Positive examination	No.	%	No.	%	p Value
P. falciparum	11	27	20	26	NS
P. falciparum only	7	17	15	19	NS
D. perstans	15	38	15	19.5	0.05*
L. loa	3	8	2	2.5	NS
Filaria	18	46	17	22	0.02†
Filaria only	14	35	12	15	0.05‡
Both plasmodium and filaria	4	10	5	6	NS
Positive for any parasite	21	52	27	35	0.1§
Negative for all parasites	15	37	45	58	0.1

NS, Not significant.

women was analyzed by means of Western blot. At birth, the infants showed the same Western blot pattern as their mothers, while at 6 months 35 of 44 (79.5%) infants were seronegative and nine (20.5%) still had antibodies to the gag gene products but not the env gene products. At 1 year of age, all the infants were seronegative in the ELISA and Western blot (as in the control group) and no Western blots were indeterminate. These findings reflect the gradual lost of maternal anti-HTLV-I immunoglobulin G.

Comment

Among the 45 HTLV-I-seropositive women studied, 38 (84.4%) were from tribes of the equatorial forest and 5 of 45 (11.1%) from tribes of the savannah. The higher seroprevalence rate among people living in the tropical rain forest have previously been described in Gabon⁹ and Zaire¹² and has been confirmed by the finding of clusters of TSP in the tropical forests of Zaire13 and Gabon.14

Pregnancy normally affects cellular immune status, with a decrease in the number of helper T lymphocytes and sometimes an increase in the number of suppressor T lymphocytes. Pregnancy may thus cause a further decline in an immune system already compromised by retroviral infection and vice versa.15 The effect of the human immunodeficiency virus on the course and outcome of pregnancy remains controversial.16

To our knowledge, the only described case of adult T-cell leukemia in pregnancy was reported by Ohba et al.8 in 1988, in a 28-year-old Japanese woman at 38 weeks' gestation. The infant recovered and remained

^{*} χ^2 = 4.6; 95% confidence interval, 18.24 to 18.75.

 $[\]dagger \chi^2 = 6.51$; 95% confidence interval, 23.82 to 24.18.

 $[\]pm \chi^2 = 4.25$; 95% confidence interval, 17.12 to 17.62.

 $[\]S\chi^2 = 3.22.$

 $^{\|\}chi^2 = 3.08.$

Table V. Blood parasite examination and ethnic group

		Ethnic				
Blood parasites		K	B an	id other		Total (No.)
	No.	%	No.	%	p Value	
Positive only for P. falciparum	20	19.5	2	13.5	NS	22
Positive only for other parasites (one or more)	40	39.2	6	40	NS .	46
Positive for both plasmodium and other parasites	7	6.8	1 .	6.5	NS	8
Negative for all parasites	35	34.5	6	40	· NS	41
All patients	102	100	15	100		117

NS, Not significant.

free of HTLV-I infection after 3 months of life, but the mother died 6 months after delivery. In our prospective study of 45 pregnant women who seropositive for HTLV-I, none presented clinical features of HTLV-I infection either during pregnancy or in the year after delivery. Both the seropositive women and seronegative controls showed numerous anomalies in the peripheral blood cell count, including anemia, neutropenia, and eosinophilia, characteristic of communities exposed to intermittent viral and bacterial infections; chronic helminthic infection; and recurrent malaria.

However, no hematologic changes characteristic of HTLV-I infection were found, in particular rosette-like lymphocyte nuclei; the eosinophilia observed was not correlated with HTLV-I infection and is presumed to be a response to chronic helminthic infections. No conclusions can be drawn concerning the influence of pregnancy on HTLV-I infection, because we did not study a nonpregnant infected group and markers of immune status were not included in the analysis. As the onset of acute adult T-cell leukemia-lymphoma during pregnancy is rare and HTLV-I infection is a long-term condition in which the majority of the population remain asymptomatic for many years, a survey of a larger population may lead to different conclusions.

The course and outcome of pregnancy were not significantly influenced by the seropositivity of the mother, but the cases did tend to have a higher rate of preterm delivery and complicated pregnancy than controls. The lack of statistical significance could be due to the small sample size.

Although the transmission of HTLV-I has been discussed by several authors, ¹⁷⁻¹⁹ mainly in Japan, data are lacking in Africa. The main route of transmission seems to be mother to child, as suggested by familial clustering ¹⁷ and surveys of seropositive mothers and their children. ¹⁸ Geographic and familial clustering of HTLV-I is also common in Gabon. The vertical route of transmission is naturally limited to three possible modes: transplacental, intrapartum, and through the maternal milk. ^{19, 20} As HTLV-I is not endogenous, ge-

netic transmission is unlikely. A follow-up study of the mothers and their infants is in progress. After 1 year, none of the children we examined had undergone seroconversion to HTLV-I, suggesting that there was no intrauterine infection. However, a longer follow-up of these children and their siblings is needed to evaluate mother-to-child transmisson, including transmission through milk, because serconversion usually occurs beyond the first year of life.²⁰

Mother-to-child transmission is estimated to occur in approximately 20% of breast-fed children and 1% to 4% of bottle-fed infants in Japan, although results are not uniform. In Japan, seroconversion occurs in 25% of the children of seropositive mothers after 3 years of life 19, 20 and in 50% of children if they are breast-fed, 20 even if only for a short period. By contrast preliminary results from a study in Haiti²¹ indicate that only 12% of infants have had seroconversion by the age of 18 to 34 months. It will be interesting to compare the results from Japan and Haiti with those of our cohort. Further investigations are necessary to evaluate what factors could influence the relative importance of this mode of transmission.

Although transmission can occur by the transfusion of infected blood products, no history of transfusion was found in either group of subjects we studied.

Sexual transmission has been incriminated by several authors. ^{22, 23} In Japan, HTLV-I seroprevalence is higher in women >30 than in men of the same age, suggesting that transmission occurs predominantly from the man to the woman. ^{3, 22} In Gabon, where prevalence increases with age but is sex independent, ⁹ syphilitic serologic results, the number of sexual partners, and the number of abortions adjusted for age are good indicators of sexual activity, because contraception is uncommon and the prevalence of sexually transmitted disease is high. ¹⁰

In our study sexual transmission did not seem to be a major route. In a recent study of female prostitutes in Kinshasa, Zaire,¹³ no association between HTLV-I seropositivity and the number of partners was observed, as in our study, suggesting that sexual trans-

mission of HTLV-I is less efficient than sexual transmission in HIV-1 infection. Nevertheless, that study showed that prostitution is a risk factor and that sexual transmission of the virus does occur. In Jamaica, women with a high cumulative number of sex partners and serologic evidence of syphilis were more likely to be HTLV-I seropositive.²³

The involvement of arthropods in the transmission of HTLV-I has been suggested²⁴ but remains controversial²⁵; in our study the rate of filaria infection was significantly higher in the HTLV-I-seropositive group than in the controls, but this finding must be interpreted with caution. Indeed, although the women in both groups lived in Franceville in a similar environment, their relative exposure to insect vectors may be different when they visit their village of origin.

In conclusion, HTLV-I seropositivity in pregnant women did not affect the course or outcome of pregnancy in comparison with a noninfected control group. There was no evidence of mother-to-child transmission at 12 months of infant life, although follow-up of this cohort is continuing. HTLV-I infection and filaria infection were correlated, but this may be due to confounding factors that were not investigated here.

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Detection of *Chlamydia trachomatis* endocervical infection in asymptomatic and symptomatic women: Comparison of deoxribonucleic acid probe test with tissue culture

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A deoxyribonucleic acid probe assay (PACE 2, Gen-Probe, San Diego) was compared with a standard tissue culture method for detection of *Chlamydia trachomatis* endocervical infection in both asymptomatic and symptomatic women. The results of the probe test were expressed as a ratio of relative light units of the specimen per relative light units of the cutoff recommended by the manufacturer. Samples with sample/cutoff ratios near 1.0 were repeated until two or more consistent ratios were obtained. A total of 426 specimens were obtained, with an overall disease prevalence of 10.1%. Of the 426 specimens examined, seven (1.6%) were near the cutoff and were retested. The results of 426 samples with matching cultures indicated that the manufacturer's discrete cutoff was adequate for results determination. The deoxyribonucleic acid probe test was essentially equivalent to standard tissue culture in terms of sensitivity, specificity, and positive and negative predictive values in a low-prevalence patient population. (AM J OBSTET GYNECOL 1991;165:1444-53.)

Key words: Chlamydia trachomatis, deoxyribonucleic acid probe, tissue culture

Infection by *Chlamydia trachomatis* is recognized as the most prevalent sexually transmitted disease worldwide, with an estimated four million cases per year in the United States,^{1, 2} While *C. trachomatis* is an important pathogen in lower and upper genital tract infections and pregnancy complications, a substantial number of cases occur without symptoms in patients.^{1, 3, 4} The most important factor in controlling the chlamydia epidemic is correct and timely diagnosis. This has previously been limited to cell culture, which is considered the gold standard in spite of <100% sensitivity. New techniques include antigen detection with enzyme immunoassay, direct fluorescent antibody, and deoxyribonucleic acid (DNA) probe tests.

PACE 2 (Gen-Probe, San Diego) is a commercially available, rapid detection test for *C. trachomatis* endocervical and urethral infection. The PACE 2 system uses the technique of nucleic acid hybridization to identify *C. trachomatis* DNA directly from urogenital swab specimens. Nucleic acid hybridization tests are based on the

ability of complementary nucleic acid strands to specifically align and form stable double-stranded complexes. PACE 2 uses a single-stranded DNA probe labeled with a chemiluminescent marker. The probe is complementary to ribosomal ribonucleic acid (rRNA) of the target organism. If rRNA from C. trachomatis is present in a collected specimen, the labeled DNA probe combines with it to form a stable DNA/RNA hybrid. Magnetic spheres are added to the reaction and these bind only to the stable hybrids. The labeled hybrids are magnetically separated from the nonhybridized probe and measured in a luminometer. A greater light response is generated with increasing numbers of stable hybrids present in the specimen (Fig. 1). The luminometer and magnetic separation unit are the only specialized laboratory equipment needed to perform this test. The reagents are commercially available from Gen-Probe. Other materials required include water bath, vortex mixer, micropipettes, and pipettes that are routinely available in most clinical laboratories.

The purpose of this study was to compare a new DNA probe assay format, designed to increase sensitivity and simplify procedural steps, with a standard cell culture method for the detection of *C. trachomatis* in endocervical swab specimens in both asymptomatic and symptomatic women seen for obstetric and gynecologic care. Currently, chlamydia infection may remain undiagnosed, misdiagnosed, or empirically treated with antibiotics because the standard diagnostic test, namely, cell culture, remains unavailable in smaller hospitals, is

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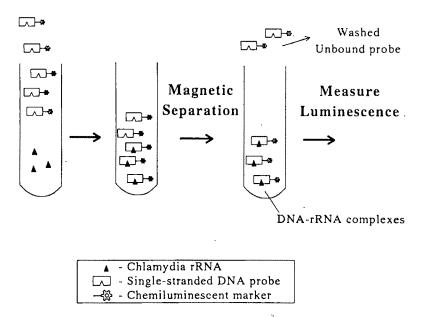


Fig. 1. Nucleic acid hybridization test for the identification of C. trachomatis DNA from urogenital swab specimens.

labor intensive, and requires a lengthy turnaround time. The development of a reliable, rapid, and easyto-use laboratory test for the detection of chlamydial infection has important ramifications for patients and clinicians. We also evaluated whether the discrete manufacturer-recommended cutoff value is sufficient for unequivocal reporting of results for all patient specimens.

Methods

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Patient population. Patients in the study were divided into two groups. Group 1 consisted of asymptomatic pregnant women who were seen for prenatal care. Patient charts were reviewed to determine the presence or absence of symptoms related to chlamydial infection at the time of specimen collection. Group 2 consisted of women seen for gynecologic care with symptoms of lower genital tract infection (i.e., vaginal discharge, pelvic pain, dyspareunia, coexisting sexually transmitted disease), patients requesting cultures because of known sexually transmitted disease exposure, and pregnant women with symptoms. Patients receiving antibiotics (duration, ≤4 weeks) before specimen collecton and individuals with no clinical histories were excluded from the study. All patients were seen at Good Samaritan Hospital Outpatient Clinic or Group Health Associates, Cincinnati, between September 1989 and June 1990.

Specimen collection. The exocervix was first cleansed of excess mucus with a Dacron swab. Subsequently, two endocervical specimens were obtained, one for DNA probe test and one for C. trachomatis culture. The samples were collected in no designated order, and

collection was not randomized. However, collection was done by 17 different physicians, thus avoiding systematic collection bias. Specimens collected for DNA probe test were placed in Gen-Probe transport tubes containing 1.0 ml of specimen preservative and glass beads. Samples were stored at room temperature for up to 1 week before being tested. For culture, specimens were placed in chlamydia transport media (2-sucrose-phosphate media, Bartel's Immunodiagnostic Supplies, Bellevue, Wash.) and processed immediately or stored at 4° C for no longer than 24 hours.

DNA probe assay. The Gen-Probe PACE 2 system chemiluminescent-labeled DNA probe test was used in the study. This system uses an acridinium-ester-labeled single-stranded DNA probe that is complementary to ribosomal rRNA of C. trachomatis.5

All reagents and samples were brought to room temperature before assay. Each sample was mixed in a vortex for 10 to 15 seconds. Swabs were then expressed of liquid on the side of the tube and discarded. Reconstituted probe reagent (100 µl) was pipetted into each tube. Specimens were mixed in a vortex, and then 100 µl of sample was added to the probe. The tubes were covered with a sealing card and the tube rack was shaken three to five times to mix. The mixture was incubated at 60° ± 1° C water bath for 1 hour. One milliliter of separation solution was added to each tube and then incubated at 60° ± 1° C for 10 minutes. The separation rack was placed onto a magnetic base at room temperature for 5 minutes.

The supernatant was decanted and the tubes blotted on absorbent paper. Each tube was filled to the rim with wash solution. The tubes remained on the separation

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rack for 20 minutes at room temperature The supernatant was then decanted but not blotted. The tube rack was shaken to resuspend the pellets. For each set of specimen runs, three negative controls and one positive control were simultaneously processed.

The tubes were read on the Leader I luminometer (Gen-Probe) with the chlamydia protocol selected. The results of the test were calculated based on the difference between the response relative light units (RLU) of the specimen and the mean relative light units of the three negative references.

Culture. Specimens were mixed in a vortex with sterile glass beads for 60 seconds, and debris was removed by centrifugation at 900 g for 5 minutes. Supernatant (200 µl) was inoculated into each of two shell vials containing McCoy cell monolayers on coverslips (Bartel's Immunodiagnostic Supplies, Bellevue, Wash.). Vials were centrifuged for 1 hour at 3000 g at 25° C. Supernatant was aspirated, and 1.0 ml of chlamydia culture media with cycloheximide was added to the monolayers. Vials were incubated at 35° C for 48 hours. The coverslip from one vial was fixed in chilled acetone and stained with fluorescein-labeled antichlamydial mouse monoclonal antibody reagent (Bartel's). Coverslips were examined at a magnification of ×100 and inclusions were confirmed at ×400 with an Olympus fluorescent microscope. A culture was considered positive if one or more inclusions were present. Passage of the second vial was performed on questionable one-vial results, monolayers showing elementary bodies but no inclusions, toxic reactions, or any specimen with discrepant probe and culture results.

Discrepant results. All specimens were stored at -70° C for further reference if discrepant results occurred. When discrepant results were obtained, specimens were submitted in blind fashion to Gen-Probe for analysis by probe competition assays. The samples were tested with the PACE 2 assay format with halfvolume reactions (50 μl sample and 50 μl probe). Three separate assays were performed for each specimen: (1) sample and labeled C. trachomatis probe, (2) sample and labeled C. trachomatis probe and ×100 unlabeled homologous probe (C. trachomatis), and (3) sample and labeled C. trachomatis probe and ×100 unlabeled heterologous probe (Mycoplasma pneumoniae). A 90% reduction in difference expressed in relative light units (competition) in tube No. 2 versus tubes No. 1 and No. 3 indicated that a specific hybridization reaction took place and that C. trachomatis nucleic acids were present in the sample. A reduction <90% indicated that a specific hybridization reaction had not taken place (no competition) and that C. trachomatis nucleic acids were not present in the sample (Fig. 2). In addition, DNA probe assays were performed on all specimens submitted for culture when culture was positive and DNA probe assay was negative.

Cutoff range for the DNA probe assay. The results of the DNA probe test were calculated on the basis of the difference between the response in relative light units of the specimen and the mean of three negative reference values. According to the manufacturer, the specimen should be considered positive for C. trachomatis if the difference between the specimen response and the mean of the negative reference is ≥ 300 RLU. One of the objectives of the study was to determine if using such a discrete manufacturer recommended cutoff value is sufficient for unequivocal reporting of results for all patient samples. This question is especially important for patient samples that may have values in relative light units that are close to the recommended cutoff value. Therefore we obtained interassay precision data for samples at various levels from the cutoff value, examined the distribution of values in relative light units for all patient samples in relation to the cutoff value, and correlated the DNA probe test results with those of tissue culture and clinical data. All patient values in relative light units were expressed as a ratio of the sample relative light units to the cutoff relative light units recommended by the manufacturer. Ratios ≥1.0 represented patient specimens that were positive for C. trachomatis (i.e., difference ≥ 300 RLU). Ratios <1.0 represented patient specimens that were negative for C. trachomatis (i.e., difference <300 RLU). Additionally, more decisive negative or positive results were obtained the further a given ratio deviated from the value of 1.0. Specimens with ratios near 1.0 were multiply tested to determine if a cutoff range rather than a discrete cutoff point could be determined for more accurate results.

Statistics. Nonparametric comparisons of categoric variables used χ^2 analysis. Calculations for test validity were made with standard formulas for sensitivity, specificity, and predictive values.

Results

A total of 426 endocervical specimens were evaluated by tissue culture and by the Gen-Probe PACE 2 DNA probe assay. An additional 2703 patient specimens without matching cultures were analyzed by DNA probe only. These additional samples were used to obtain more precise analysis of borderline specimens and to verify the distribution of study samples around the cutoff.

Interassay precision data. Table I shows betweenrun precision data for the DNA probe assay for matched and unmatched specimens. This interassay precision data was calculated by assaying selected samples in several consecutive runs. A negative control sample, a positive control sample, and several patient samples with various sample/cutoff ratios were evaluated. The coefficient of variation for the negative control samples was approximately 28%, whereas the coeffi-

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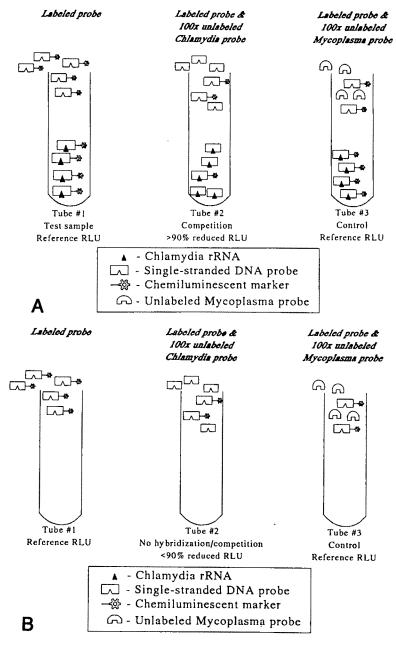


Fig. 2. Probe competition assay. A, Positive reaction occurred when >90% reduction in difference in relative light units was detected in tube No. 2 compared with tubes No. 1 and No. 3. When C. trachomatis nucleic acids were present in the sample, competition reaction occurred in tube No. 2. B, Negative reaction occurred when <90% reduction in difference in relative light units was detected in tube No. 2 compared with tubes No. 1 and No. 3. No competition reaction occurred in tube No. 2.

cient of variation for the positive control samples was approximately 16%. The coefficients of variation for the patient samples were as high as 23%. With such interassay variation of up to 30%, occasional patient samples that were truly positive for C. trachomatis could, on a single run, have a ratio as low as 0.7. Patient samples near the cutoff value (ratio 1.0) were assayed several times to ascertain whether DNA probe retesting of those samples allowed for consistent and clinically relevant results. Samples were considered negative for

C. trachomatis by the DNA probe assay if results were consistently < 1.0 (i.e., $0.7 \le \text{ratio} < 1.0$) or a single ratio ≥ 1.0 (i.e., $3.0 > \text{ratio} \geq 1.0$) occurred among other results < 1.0. Samples were considered positive for C. trachomatis by the DNA probe assay if results were consistently ≥ 1.0 (i.e., $3.0 > \text{ratio} \geq 1.0$) or a single ratio < 1.0(i.e., $0.7 \le \text{ratio} < 1.0$) occurred among other results $\geq 1.0.$

Correlation of the DNA probe assay results with those of culture and clinical data. In this study, true

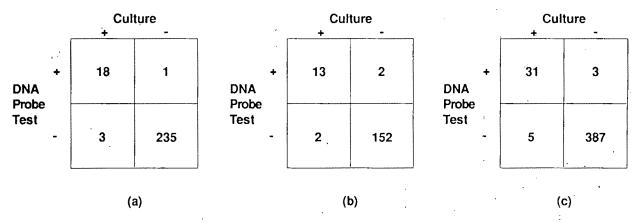


Fig. 3. Comparison of DNA probe test and tissue culture for detection of *C. trachomatis.* a, Pregnant patients without symptoms; b, patients with symptoms; c, combined patients with and without symptoms. True-positive samples were those specimens positive by tissue culture or by two nonculture tests (i.e., DNA probe test and probe competition assay) if tissue culture was negative.

Table I. Interassay precision data

Sample	No. of DNA tests run	Sample cutoff (RLU)		
		Mean	· SD	Coefficient of variation (%)
Negative control	22*	0.29	0.08	27.6
Positive control	$\frac{\overline{21}}{21}$	4.32	0.70	16.2
Patient identification No.				, .
36764	5	0.39	0.18	19.4
35128	5 ·	0.46	0.08	17.4
31731	6	0.82	0.19	23.2
34399	4	1.16	0.17	14.7
30888	6	2.47	0.12	4.8
35322	5	5.41	0.43	8.0
37775	4	6.86	0.40	5.8
39529	5	9.06	0.69	7.6
35178	5 .	15.75	1.62	10.3
38897	5	33.20	2.30	6.9

^{*}Two negative controls had values >3 SDs from the mean (negative control No. 1 had a ratio of 0.67; negative control No. 2 had a ratio of 0.63), and they were not used to produce these results.

positive samples were considered to be those specimens that were positive by culture or positive by two non-cultural tests (i.e., DNA probe test and probe competition assay) if culture was negative. The results obtained by DNA probe and culture for asymptomatic and symptomatic patients are shown in Fig 3, a and b, respectively. Fig. 3, c, depicts the data for all patients, regardless of symptoms.

The number of pregnant patients without symptoms included in the study was 257 (Fig. 3, a). Twenty-two patients had positive results by culture alone or two noncultural methods, resulting in a disease prevalence of 8.6%. Four of these patients had discrepant results. One patient was DNA probe positive, but culture negative. This sample was positive for *C. trachomatis* after repeat DNA probe testing (i.e., ratios, 8.35, 9.32). The sample for DNA probe was also submitted in blind fashion for probe competition assay, which verified the

presence of *C. trachomatis* nucleic acid. The three remaining patients were all culture positive and DNA probe negative. The negative results were confirmed by DNA probe competition assay. These probe samples were also retested with the DNA probe assay and all resulted in negative ratios (i.e., patient 42315, ratios = 0.51, 0.45, and 0.28; patient 35543, ratios = 0.17 and 0.25; patient 39971, ratios = 0.48 and 0.37). DNA probe assays performed on the specimens submitted for culture were all positive. The DNA assay demonstrated 98.4% correlation with culture in asymptomatic patients.

The number of symptomatic patients was 169. Seventeen patients had positive results by culture alone or two noncultural methods, with a disease prevalence of 10.1%. Four of these patients had discrepant results (Fig. 3, b). Two patients were positive by culture and negative by DNA probe test. The samples submitted

Table II. Sensitivity, specificity, and predictive values for DNA probe and culture

Group	Sensitivity				Negative predictive value		
	%	95% Confidence interval	Specificity (%)	Positive predictive value (%)	%	95% Confidence interval	
Without symptoms		., .				<u> </u>	
DNA probe test	86.4	75-100	100	100	98.7	97-100	
Culture	95.4	87-100	100	100	99.6	99-100	
With symptoms							
DNÁ probe test	88.2	76-100	100	100	98.7	97-100	
Culture	88.2	76-100	100	100	98.7	97-100	
Combined							
DNA probe test	87.2	79-98	100	100	98.7	98-100	
Culture	92.3	85-100	100	100	99.2	98-100	

Table III. Sensitivity, specificity, and predictive values for DNA probe versus culture alone as gold standard

		Sensitivity		Specificity		Positive predictive value		Negative predictive value	
DNA probe test group	%	95% Confidence interval	%	95% Confidence interval	%	95% Confidence interval	%	95% Confidence interval	
Without symptoms Symptomatic Combined	85.7 86.7 86.1	71-100 70-100 75-97	99.6 98.7 99.2	99-100 97-100 98-100	94.7 86.7 91.2	85-100 70-100 82-100	98.7 98.7 98.7	97-100 97-100 98-100	

for the DNA test were subsequently retested with the DNA probe assay. The DNA probe assay was consistently negative for both samples (i.e., patient 37640, ratios = 0.44 and 0.47; patient 28725, ratios = 0.41and 0.38). The probe competition assays verified the absence of C. trachomatis nucleic acid in the specimens submitted. DNA probe assays performed on the specimens submitted for culture were positive. The two remaining patients were negative for C. trachomatis by culture and positive by DNA probe test. The DNA probe test was performed several times on these probe samples and consistently positive results were obtained (i.e., patient 29983, ratios = 1.31, 1.54, and 1.28; patient 26549, ratios = 3.14, 3.56, and 3.58). Probe competition assay confirmed the presence of C. trachomatis nucleic acid in the probe samples. The DNA probe assay demonstrated 97.6% correlation with culture in symptomatic patients.

Fig. 3, c, shows the statistical parameters for the DNA probe test and culture when the results from the patients with and without symptoms were combined. Of the total 426 patients tested, C. trachomatis was detected in 36 (8.4%) specimens by culture and in 34 (8.0%) specimens by the DNA probe test. Overall, the DNA probe test demonstrated 98.1% agreement with culture. For all patients combined, the disease prevalence was 10.1%. There was no statistical difference between DNA probe test and standard cell culture results by χ^2 analysis.

Table II depicts the sensitivity, specificity, positive

and negative predictive values, and 95% confidence intervals for both the DNA probe test and culture in asymptomatic, symptomatic, and combined patient groups using culture or two noncultural methods as the gold standard. Table III depicts the sensitivity, specificity, positive and negative predictive values, and 95% confidence intervals for the DNA probe versus culture alone as the gold standard. Similar results were obtained for both methods of analysis.

Samples that tested negative for C. trachomatis by the DNA probe assay. Table IV shows the distribution of the sample/cutoff ratios for samples that tested negative for C. trachomatis by the DNA probe assay. With the manufacturer's recommended cutoff, 392 of 426 patients in this study were negative for chlamydia. Approximately 96% of these negative samples were at least 50% away from the cutoff value (ratio, ≤0.5). Randomly selected samples from patients with ratios ≤ 0.5 were assayed several times and remained negative (data not shown). Seventeen study patients who were negative for C. trachomatis by the DNA probe test had sample/cutoff ratios >0.5 and <1.0. Sixteen of these patients were negative for C. trachomatis by tissue culture, and only one patient was positive. Repeat testing of this patient by DNA probe assay was performed on the sample submitted for the DNA probe testing. This sample yielded consistently negative results for C. trachomatis (i.e., patient 27348, ratios = 0.53, 0.41, and 0.24). Additionally, probe competition assay performed on this sample confirmed the lack of C. trachomatis nucleic

Table IV. Distribution of values in relative light units in patients who tested negative for *C. trachomatis* by DNA probe assay

		(both DNA assay ulture)	Additional patients† (DNA assay only)		
Range (sample/cutoff)	No.	%.	No.	. %	
1.0 >Ratio ≥0.9	0		11 .	0.4	
0.9 >Ratio ≥0.8	4	1.0	· 15	0.6	
0.8 >Ratio ≥0.7	1	0.3	26	1.1	
0.7 >Ratio ≥0.6	6	1.5	32	1.3	
0.6 >Ratio ≥0.5	6	1.5	59	2.4	
0.5 >Ratio ≥0.4	9	2.3	90	3.7	
0.4 >Ratio ≥0.3	27	6.9	204	8.3	
0.3 >Ratio ≥0.2	104	26.5	677	27.7	
0.2 >Ratio ≥0,1	230	58.7	1296	52.9	
0.1 >Ratio ≥0.0	5	1.3	38	1.6	
TOTAL	392	100	2448	100	

^{*}A total of 426 study patients were evaluated for the presence of *C. trachomatis* by DNA probe assay and culture. With the manufacturer's recommended cutoff, 392 (92.0%) of the study patients were negative for *C. trachomatis* by the DNA probe assay. †A total of 2703 patients were evaluated for the presence of *C. trachomatis* by DNA probe assay alone. With the manufacturer's recommended cutoff, 2448 (90.6%) of the patients were negative for *C. trachomatis*.

acid in the sample submitted. The DNA probe assay performed on the specimen submitted for culture was positive.

An additional 2703 patient samples were analyzed by DNA probe assay alone during October 1989 and June 1990. The sample/cutoff ratios were determined (Table IV). No matching cultures were performed. Out of 2703 samples analyzed, 2448 (90.6%) specimens were negative for C. trachomatis. Approximatey 5% of patients who tested negative for C. trachomatis had sample/cutoff ratios between 0.5 and 1.0. Eleven patients had ratios >0.9 and <1.0 after initial testing. Nine of these patients were retested by the DNA probe assay. Six patients remained negative for C. trachomatis; however, three of the nine patients were positive for C. trachomatis (i.e., patient 39891, ratios = 0.96, 1.24; and 1.72; patient 33767, ratios = 0.90, 1.08, and 1.00; patient 33342, ratios = 0.98, 1.10, and 1.15). Multiple samples from patients with ratios between 0.8 and 0.9 and between 0.7 and 0.8 were assayed several times. Although all of these samples remained negative after retesting, one sample that had a ratio of 0.77 on primary testing had a single positive ratio when assayed several times (i.e., patient 29435, ratios = 0.77, 1.42, 0.99, 0.67, and 0.55).

Samples that tested positive for C. trachomatis by the DNA probe assay. Table V depicts sample/cutoff ratios in patient samples that tested positive for C. trachomatis by the DNA probe assay. The ratios are shown for all study patients (patients who had a DNA probe assay and a matching culture) and for 2703 patient samples evaluated only by the DNA probe assay without matching cultures. Randomly selected patients with sample/cutoff ratios ≥ 3.0 were retested for C. trachomatis by the DNA probe assay, and all remained positive

(data not shown). Two study patients had sample/cutoff ratios <3.0 and ≥ 1.0 . One patient (i.e., patient 24537, ratio = 2.47) was positive for *C. trachomatis* by culture. The other patient tested negative by culture; however, this patient was clinically symptomatic and was positive for *C. trachomatis* by repeat DNA probe testing on the probe specimen (i.e., patient 25897, ratios = 1.3, 1.5, 1.2). Additionally, probe competition assay confirmed the presence of *C. trachomatis* nucleic acids in this patient's sample.

Of the 2703 patients who had only the DNA probe test performed, 38 (1.4%) had sample/cutoff ratios <1.5 and ≥1.0 . Twenty-six of these patients were retested for *C. trachomatis* by DNA probe assay. Eight patients remained positive for *C. trachomatis*, whereas 17 had negative ratios.

In the same group of 2703 patients, 14 (0.5%) had sample/cutoff ratios <2.0 and \ge 1.5. Eight of these patient samples were retested by the DNA probe assay. Three remained positive for *C. trachomatis*, whereas five had negative ratios after retesting.

Finally, of the 2703 patients who had only the DNA probe test performed, 13 (0.5%) had sample/cutoff ratios <3.0 and ≥ 2.0 . Five of these patient samples were retested by the DNA probe assay, resulting in only one patient with negative ratios for *C. trachomatis* (i.e., patient 29987, ratios = 2.05, 0.46, and 0.98). Randomly selected samples from patients with ratios ≥ 3.0 were assayed several times; all samples remained positive (data not shown).

Comment

Infection by *C. trachomatis* is limited almost exclusively to humans.⁶ It is one of the most prevalent pathogens in the world and is the most common sexually

Table V. Distribution of values in relative light units in patients who tested positive for C. trachomatis by DNA probe assay

		(both DNA assay culture)	Additional patients† (DNA assay only)		
Range (sample/cutoff)	No.	%	No.	%	
Ratio ≥10.0	26	76.5	137	53.7	
10.0 >Ratio ≥7.0	2	5.9	10	3.9	
7.0 >Ratio ≥6.0	1	2.9	9	3.5	
6.0 >Ratio ≥5.0	0 .	0.0	10	3.9	
5.0 >Ratio ≥4.0	0	0.0	8	3.1	
4.0 > Ratio ≥ 3.0	3	8.9	16 .	6.3	
3.0 >Ratio ≥2.0	1	2.9	13	5.1	
2.0 >Ratio ≥1.5	0	0.0	14	5.5	
1.5 >Ratio ≥1.0	1	2.9	38	14.9	
TOTAL	34	100	255	100	

^{*}A total of 426 study patients were evaluated for the presence of C. trachomatis by DNA probe assay and culture. With the manufacturer's recommended cutoff, 34 (8.0%) of the study patients were positive for C. trachomatis by the DNA probe assay.

transmitted disease in developed countries. Cell culture has remained the standard for diagnosis, although some studies have estimated that a single endocervical specimen may be only 70% to 90% sensitive for C. trachomatis infection.7.8 Cell culture is technically difficult and requires organism viability and timely transport of specimens to the laboratory for processing. In addition, cell culture is labor intensive, requires trained personnel, and has a routine turnaround time of 48 hours. Demand for simplified and rapid methods of diagnosis has led to development of many antigen detection methods for C. trachomatis infection over the past 10 years. These methods are based on immunodetection of solubilized chlamydia components (enzyme immunoassay) or direct visualization of organisms with chlamydia-specific fluorescein-conjugated monoclonal antibodies (direct fluorescent antibody). Studies comparing these noncultural methods to standard cell culture report sensitivities of enzyme immunoassay of 67% to 98% and sensitivities for direct fluorescent antibody of 72% to 99%.7-10 Barnes6 recently reviewed several studies from 1984 to 1988 comparing enzyme immunoassay with cell culture. These studies demonstrated a range of sensitivity from 70% to 100% and a positive predictive value from 6% to 92%. The prevalence of endocervical infection ranged from 5% to 40%. Similar comparison of direct fluorescent antibody to cell culture in populations with a prevalence of 5% to 27% showed sensitivities from 56% to 100% and positive predictive values from 47% to 100%. Because of the wide range of sensitivities reported in many studies, antigen detection tests have met with mixed acceptance by clinicians for the detection of chlamydial infection. Recently, attention has turned to DNA probes for the detection of infection, and favorable results have been reported with sensitivities of 92%.11

Our study population had a prevalence of infection of 8.6% and 10.1% for asymptomatic and symptomatic patients, respectively. The prevalence of infection in asymptomatic women is comparable to previous reports of 7% to 11%.7, 11, 12 The infection rate in the symptomatic group is lower than expected but comparable to previous reports of 14% to 20%. 9, 11 Our symptomatic group probably does not represent a truly high-risk subpopulation. The DNA probe test performed well in our relatively low-prevalence population.

Comparison of nonculture tests, such as the DNA probe, with culture methods of <100% sensitivity must be interpreted with caution. If culture alone is used as the gold standard, then culture would necessarily have 100% sensitivity. Positive DNA probes might be misinterpreted as being falsely positive rather than culture results being falsely negative. We attempted to address this issue by sending discrepant specimens to another laboratory for DNA probe competition assays. Probe competition assays are reported by Gen-Probe as having 100% correlation with other hybridization techniques. Our results were blinded to the outside laboratory. In this study we defined the number of true positives to be the sum of specimens positive by culture or positive by a DNA probe assay, with confirmation of the presence of C. trachomatis nucleic acid in the sample by probe competition assay. Although this is not standard analysis, other authors have attempted to address this issue similarly with noncultural method confirmation.7-9 We did include standard analytic parameters of test validity with culture alone used as the gold standard (Table III), and, comparable results for the DNA probe were obtained. Overall, the DNA probe performed well with 98% agreement with culture in our study.

In the asymptomatic population, three patients were

[†]A total of 2703 patients were evaluated for the presence of C. trachomatis by DNA probe assay alone. With the manufacturer's recommended cutoff, 255 (9.4%) of the patients were positive for C. trachomalis.

DNA probe negative but culture positive for *C. trachomatis*. DNA probe competition assay was negative in all these samples, indicating that rRNA for *C. trachomatis* was not present in the specimens submitted for the DNA probe assay. DNA probe tests done on the culture-positive specimens were also positive, indicating the presence of probe-detectable DNA sequences. The one patient who was positive for *C. trachomatis* by DNA probe test and negative by culture was found to have *C. trachomatis* nucleic acid present in the probe sample by probe competition assay. The DNA probe assay gave no false-positive results in this patient population.

We obtained similar results with our patient population with symptoms. Two patients were DNA probe negative and culture positive. DNA probe competition assays did not indicate the presence of *C. trachomatis* rRNA. Again, DNA probe tests done on culture-positive specimens were positive indicating the presence of probe-detectable DNA sequences. Two patients were DNA probe positive and culture negative. Probe competition assays verified the presence of *C. trachomatis*; therefore these samples were considered true positives. Overall, the tissue culture method produced three false-negative results, whereas DNA probe assay produced five false negatives.

Tissue culture technique can have a large impact on the ability to isolate *C. trachomatis*. Single passage with shell vials and immunofluorescence staining is standard technique and has been shown to yield high sensitivities. ¹³ Automatic passage of tissue culture specimens has been reported to increase the sensitivity of this method by approximately 3% to 10%. ^{6,14} However, passage with this technique is not commonly practiced in many large clinical institutions. Multiple passages in shell vial systems, in contradistinction to microtiter plates, has not been shown to increase sensitivity of culture. ¹⁴ For these reasons, automatic passage was not done on tissue culture specimens in this study. All discrepant or suspicious specimens underwent a second passage with confirmation of initial results.

Sampling error is the most likely source of discrepant results for both culture and DNA probe assay. Patient specimens for culture and for the DNA probe test were collected with separate swabs. In previous culture versus nonculture evaluations, discrepancy rates of 5% to 13% have been reported as a result of sample variation between multiple swabs. Detection of chlamydia by any method requires the sampling of endocervical cells. Obtaining several swabs and removal of excess mucus has been observed to increase detection rate. Detection rate.

One problem we found with the DNA probe test was the discrete manufacturer recommended cutoff of 300 RLU above the mean of the negative references. The results of 426 culture-matched samples suggested that the manufacturers' recommended discrete cutoff was adequate for determination of results. Our results on 2703 patient samples without matching cultures indicated that samples with sample/cutoff ratios of 0.7 to 3.0 should have repeat testing until unequivocal results are obtained. Out of the 3129 patient samples evaluated by the DNA probe assay in this paper, 124 patients (4.0%) fell into this range. Further study with matching cultures would be helpful to confirm this finding. However, without modification the DNA probe test was equivalent to standard tissue culture in sensitivity, specificity, and positive and negative predictive values for our 426-patient study population, when culture alone or two noncultural methods were used as the gold standard.

The fact sheet from the National Institute of Allergy and Infectious Disease in 1986 recommended that all sexually active women <35 years old be tested yearly for C. trachomatis.17 The DNA probe is a rapid, costeffective method of screening women for infection with 87.2% sensitivity in our low-risk populations. Infection by chlamydia can potentially result in serious long-term sequelae, including ectopic pregnancy, infertility, chronic pelvic pain, and pelvic inflammatory disease. Vertical transmission from mother to fetus has been well established. Recent studies implicate chlamydial infection with increased risk of premature rupture of the membranes, low birth weight, and preterm delivery.3, 4, 18 Rapid tests for diagnosis of chlamydial infection in obstetrics would be of benefit to mother and fetus. Treatment is simple and effective once the diagnosis is made with laboratory confirmation. Positive test results improve diagnostic correlation for the physician and patient treatment compliance and enhance the likelihood that her sex partner will seek referral for evaluation. Coinfecton rate with Neisseria gonorrhoeae can be as high as 50%. The Centers for Disease Control currently recommend presumptive treatment for both organisms if one is detected.19 Specific diagnosis can direct antibiotic therapy for patients and their partners. The DNA probe test also can be used for the detection of both organisms from the same endocervical sample, and results can be made available in approximately 2 hours. In addition, the DNA probe can be a costeffective alternative to cell culture. Our study indicates that the PACE 2 DNA probe is a reasonable alternative to standard cell culture for the rapid diagnosis of chlamydial infection in women. Future DNA probe technology likely will be applied to many other types of infection.

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Editors' note

The American Journal of Obstetrics and Gynecology introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case réport, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Loxoscelism threatening pregnancy: Five cases

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Envenomation by the spider *Loxosceles reclusa* in five pregnant women proved to have no sustained adverse effects on mother or baby when managed conservatively only with low-dose prednisone. A striking toxic erythema of the skin, common with the bite of the spider, caused the greatest discomfort and concern for the patients but proved to be entirely tractable. No episodes of hemolysis, disorders of coagulation, or renal damage were discovered. There is no clinical evidence of any special risk during pregnancy in the event of loxoscelism. (AM J OBSTET GYNECOL 1991;165:1454-6.)

Key words: Loxoscelism, skin disease

A North American arachnid, the brown recluse spider, properly known as *Loxosceles reclusa*, is the most poisonous house spider in the United States. ^{1, 2} As a result of being carried with household goods and warehouse cargo during the last 50 years, this spider occasionally may be found far beyond its original Midwest habitat. ³ Today, occasional diagnoses of loxoscelism are received from every segment of the nation, often from northern and eastern regions, where envenomations have not been recognized before. No prior reports have been made concerning the course of loxoscelism during pregnancy.

Although no deaths as a result of loxoscelism are fully proved and published, the potential for fatality seems evident. At worst, the bite of this tiny spider may cause an enormous necrotic ulcer of the skin, occasionally as large as 40 cm across and extending into deep muscles; or large amounts of venom may induce sudden severe hemolysis, activate complement, and produce rapid injury to the kidneys or other organs. Children seem particularly susceptible to rapid hemolytic reactions. More pleasantly, the brown recluse spider tries to avoid humans. This nocturnal creature will flee across the skin without biting, unless trapped or assaulted; it seldom occupies busy areas of the home, choosing to hide in closets or in the sides of corrugated cardboard boxes. Importantly, almost all bites are medically insignificant. Unless massive necrosis of the skin results, hematologic complications occur, or unusual apprehension develops (as in the cases reported here), then the physician's care is not essential.

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6/1/30080

This report concerns five healthy, pregnant women with medically significant, probable (three of five cases) or proved loxoscelism. The treatment and the wholly favorable outcome for the fetus are described.

The older medical literature failed to establish accurately useful rules for the diagnosis of loxoscelism.⁴ All unexplained necrotic, purple, cutaneous macules of skin are not necessarily spider bites. A physician's visual diagnosis alone—however loud or authoritative the claim—if unsupported by actual material evidence is insufficient for undeniable clinical diagnosis.⁵ Such cases are inappropriate for publication (Table I).

Case reports: Proved loxoscelism during pregnancy

Case 1. A 32-year-old woman, wife of a physician, gravida 2, para 1, 71/2 months pregnant, living in central Illinois, was bitten about noon in late June while sorting clothing and storing cardboard boxes in her basement. She felt "something" run around her waistline and "sting" at her posterior flank. She struck and killed a young Loxosceles reclusa (which was later expertly verified) on the site and saved the "bug" to show her husband. By dinnertime the site was unusually painful, showing a sunken blue macule about 1 cm round, with a small red halo. The patient felt weak and nauseated. By the next morning the pain had worsened, but much more threatening was a bright red maculopapular eruption that spread rapidly all over the skin, especially on the face, associated with the worsening of nausea and with myalgia. Consultation by telephone at 20 hours after the bite furnished these laboratory data: hematocrit, 38%; normal platelet count; white blood cell count, 11,000 cells/mm³ with 80% polymorphonuclear leukocytes; and no free serum hemoglobin. Urine was normal and, most notably, free of blood. Her local physicians opted for bed rest, padding, splinting of the area, and prednisone 40 mg daily, pending further evaluation. No worsening occurred; the trivial laboratory anomalies resolved, and the toxic diffuse erythema of the skin disappeared entirely by the third day. Prednisone was continued to the fifth day. The pregnancy seemed undisturbed, and a wholly normal fetus subsequently was delivered.

Case 2. A 27-year-old woman, gravida I, para 0, 8 months pregnant, who resided in northern Missouri, slipped into her bathrobe and was bitten painfully on the dorsal arm, near the shoulder. She killed a Loxosceles reclusa (proved later) found in her sleeve. While later receiving antihistamines from her personal physician, she became alarmed when the firm, sunken, purple area enlarged almost hourly to reach about 3 to 4 cm in diameter, with a 3 cm halo of erythema around it. When my colleagues and I were consulted almost 80 hours after the bite, the patient's feet were mottled red and swollen, but the bite area had stabilized. The laboratory data were as follows: white blood cell count, 18,000 cells/mm3 with 90% polymorphonuclear leukocytes; platelet count, normal; fibrin split products, <100 µg/100 ml; and urine, positive for free hemoglobin and protein, without any red blood cells seen in the sediment. Bed rest, splinting, elevation of the arm, and padding were started, but corticosteroids were not given because the hemolytic process was judged to be complete at almost 4 days. Recovery was rapid; however, slight proteinuria continued until after delivery. The baby was entirely healthy.

Case reports: Probable loxoscelism

Case 1. A 34-year-old woman, gravida 4, para 3, 6 months pregnant, wife of a physician, was bitten, probably during sleep, in early June. A 2 cm lesion typical of a mild brown recluse spider bite developed on the thigh. Loxosceles spiders were later recovered from the basement ceiling of her home; verified bites are very common in June in the county in which she lives. Seen 48 hours after the bite, she had hemoglobin and protein in the urine and said that she felt "nauseated and ill." The white blood cell count was 10,000 cells/mm³ with a normal platelet count. No drug therapy was started; she recovered fully and was delivered of a normal

Case 2. A 28-year-old physician's daughter, gravida 2, para 1, 6 months pregnant, has a story almost exactly that of the prior case, except that this bite was on the buttock and was a much more necrotic, deeper reaction, 3 to 4 cm across, with more severe pain. The white blood cell count was 34,000 cells/mm³ with 90% polymorphonuclear leukocytes, and the platelet count was depressed <200,000 cu/mm³ for several days. Hemolysis was not detected in serum when the patient was seen 40 hours after the bite, but traces of blood and protein were found in the urine. She took prednisone 60 mg daily for 4 days. The bite was slow to heal but resolved entirely with minimum scarring in 7 months, well after the birth of a normal infant. Her father, a surgeon, had rejected his faith in early, vigorous, wide excision of the bite only because of his daughter's pregnancy (and possibly our advice). Loxosceles spiders are plentiful in her neighborhood.

Case 3. A gravida 1, para 0, 5½ months pregnant farm wife consulted us in July out of concern for a

Table I. Suggested standards for accurate reporting of cases of North American loxoscelism

1. Proved envenomation

- The spider must be recovered immediately and in close proximity to a clinical reaction on skin.
- That spider must be identified by an experienced entomologist and kept for later verification.
- The details of the case must be complete and the patient must be followed up properly with all appropriate expert review of the diagnostic problem.
- 2. Probable envenomation (suitable only for epidemiologic studies)
 - Spiders must be found in the immediate vicinity and these must confirmed by entomologist to be Loxoceles reclusa. Typical proved brown recluse spider bites must be medically well known in the
 - The cutaneous lesion must be wholly typical of loxoscelism, as decided by clinically experienced experts.

3. Possible envenomation

The bite appears typical to experienced physicians; however, no spiders can be recovered in the immediate vicinity. Brown recluse spiders must have been found in the region and other probable or proved bites must have occurred recently.

4. Focal necrosis of the skin

Region has no or few recluse spiders, as verified by entomologists, proved recent loxoscelism is uncommon, and no loxosceles spiders can be recovered in the immediate vicinity of the patient.

generalized severe maculopapular toxic erythematous eruption and increasing general illness in the 3 days after a mild, 1 cm, typical loxosceles bite on the thigh. Laboratory values were reassuring: white blood cell count, 16,000 cells/mm⁸ with 90% polymorphonuclear leukocytes; platelets, normal; and no free serum hemoglobin. Urine had only mild proteinuria (1+) with a trace of blood. She received 40 mg prednisone daily for 3 days, which seemed to her to resolve the rash. She recovered fully and later was delivered of a normal infant.

Comment

In this short series of cases of loxoscelism in pregnancy, no fetus seemed to be harmed, although fearful anticipation was nearly overwhelming for these patients and their families-especially for family members of physicians. In any event no actual medical situation developed that ever seemed to threaten pregnancy.

The generalized toxic erythema of the skin was the single most frightening aspect of the disease. This toxic erythema is a well-known and benign part of loxoscelism and, as a rough rule, may be a favorable sign clinically, suggesting that the patient may possess some prior natural protective immunity. Often this cutaneous reaction is accompanied by some malaise, nausea, headache, and myalgia. Less commonly, persistent hypotensive and vasomotor reactions may be present, lasting at most for a week or two. The toxic rash often appears first as a reticulated erythema or cutaneous flush all over the body then may convert rapidly into a pruritic, or tender, sheet of tiny papules resembling a drug eruption or an exanthem. The rare severe forms of toxic erythema I have seen include facial edema with paresthesia of the scalp and striking edema of hands and feet. Even initially, the hands and feet may appear swollen and deep red or purple, and pain or burning may be felt. Rarely the hands may feel cold and even numb. Tips of fingers, palms, toes, and soles may peel thoroughly 10 to 20 days later, somewhat resembling the streptococcal scarlet fever seen in old photographs.

Diffuse tiny purpura, sometimes alarming in number and persistence, may be a principal feature of the toxic erythema reaction seen with loxoscelism. Because larger purpura or ecchymoses rarely may appear suddenly on hands and feet with the toxic reaction and because both gross clotting anomalies and severe thrombocytopenia can occur in severe systemic loxoscelism, the physician must be satisfied that only the common minor toxic vascular injury, and not a major coagulation disorder, is the cause of the bleeding in the skin. As much as 3 weeks after a bite, peculiar small exacerbations of these toxic purpura may be seen, without other apparent illness. Lacking the assurance of a double-blind study, I suggest that short-term corticosteroid therapy seems to stop or greatly improve this toxic vascular reaction.

The value of a conservative approach to both cutaneous and systemic loxoscelism seems to be well demonstrated in these five cases, as well as in the general literature. In my opinion there is no argument whatsoever to be made for early surgery as therapy for any type of cutaneous loxoscelism. Most brown recluse spider bites are trivial. No one can accurately predict which few bites will progress to systemic disease or to large

cutaneous necrosis. Even those bites that do progress to coagulation disorders are much better managed medically without the complicating issues and effects of surgery. Reparative surgery, about 12 weeks later, may offer some benefits for treating exceptional ulcerations, although most will heal extremely well without surgery. Costs must be considered, too.

I have also reviewed or managed several other cases of possible loxoscelism in pregnancy that could not be included in this report. Cases are few. I know of no instance of a severe coagulation disorder caused in pregnancy by envenomation by any spider or of any case in which the fetus was harmed. Pleasantly, we have never seen sudden renal failure in pregnancy from loxoscelism, as we have seen in cases with brown recluse spider bites of comparable size in children.

Corticosteroid therapy may benefit the toxic erythema and may be helpful in preventing hemolysis and renal injury, but only if given very soon after the bite, before hemolysis is complete. Corticosteroids, even if given very early, do not prevent the development of the ulcer in the skin. Many other drugs have been tried, but none have been convincingly proved to block the cutaneous ulceration.

An entomologist's expert identification of arachnids suspected of causing disease may be obtained by arrangement with my office at no cost, providing only that a clinical report is shared.

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Failure to verify high activities of polyamine oxidase in pregnancy serum

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The activity of polyamine oxidase in human pregnancy serum was measured by our new specific method with radioisotopic N¹-acetylspermine as substrate. The activity in pregnancy serum was very low and did not increase during gestation; it was not higher than that in nonpregnancy serum. The high polyamine oxidase activities previously reported by others appear to result from contamination with diamine oxidase, the level of which is high in pregnancy serum. (AM J OBSTET GYNECOL 1991;165:1457-8.)

Key words: Polyamine oxidase, diamine oxidase, pregnancy serum, N¹-acetylspermine

Polyamine oxidase is responsible for conversion of N¹-acetylspermine and N¹-acetylspermidine to spermidine and putrescine, respectively, in mammalian tissues.¹ Free spermine and spermidine also can be substrates for polyamine oxidase, but their activity is much lower than the activity with the acetylpolyamines.² Many data showing high levels of polyamine oxidase activity in human pregnancy serum have been accumulated by a single research group.³-5 In this study we reevaluated polyamine oxidase activity in human pregnancy serum using our new specific method with N¹-acetylspermine tagged with carbon 14 as substrate and failed to confirm high levels of activity.

Material and methods

Chemicals. Pargyline hydrochloride was obtained from Sigma Chemical (St. Louis); semicarbazide hydrochloride was from Kanto Chemical (Tokyo); Amberlite CG-50 (Type I, NH₄+ form) was from Rohm and Hass (Philadelphia); ACS-II scintillator cocktail was from Amersham Co. (Arlington Heights, Ill.). Other chemicals used were of the highest quality commercially available.

Polyamine oxidase assay. Serum samples were collected from 29 healthy pregnant Japanese women at 8 to 40 weeks of gestation. The sera were grouped into early (8 to 12 weeks of gestation), middle (13 to 30 weeks), and late (31 to 40 weeks) stages. Human organs were obtained from four nonpregnant female Japanese cadavers at forensic autopsy; these women were 30 to 60 years old at death. The tissues were homogenized

in 9 volumes of 5 mmol/L potassium phosphate buffer (pH 7.4) and subjected to enzyme assays.

Polyamine oxidase activity was measured by our newly established method.6 The assay mixture (total 0.5 ml) contained 0.1 ml serum or tissue homogenate, 0.05 ml pargyline, a monoamine oxidase inhibitor (final concentration 0.1 mmol/L), 0.05 ml semicarbazide, a diamine oxidase inhibitor (final concentration 1 mmol/L), 0.1 ml 0.5 mol/L potassium phosphate buffer solution (pH 7.4), and 0.18 ml distilled water. The reaction mixture was preincubated at 37° C for 10 minutes, and then the reaction was started by the addition of 0.02 ml N¹-[¹⁴C]acetylspermine (final concentration 0.1 mmol/L). After incubation at 37° C for 60 minutes, the reaction was stopped by adding 0.05 ml of 1 mol/L hydrochloric acid solution. As a blank test, the assay mixture without substrate was incubated; the radioactive substrate was added after the hydrochloric acid solution. The mixture was transferred directly to a minicolumn (0.8 \times 4 cm) of Amberlite CG-50. Then the radioactive reaction product, 3-[14C]acetamidopropanal, was eluted with 5 ml of water. The eluted sample (5 ml) was transferred to scintillation vials containing ACS-II scintillator cocktail, and the radioactivity of the contents of the vials was measured by liquid scintillation counting.

Results

Polyamine oxidase activities were carefully measured and the results are shown in Table I. Polyamine oxidase activity in pregnancy serum was very low and not higher than that in nonpregnancy serum. In addition, the levels of activity did not increase during pregnancy. The activity in retroplacental serum, however, was about twofold higher than that in nonpregnancy serum (p < 0.01). To demonstrate the validity of our assay method, we measured polyamine oxidase activity in tissues of nonpregnant women. The activities in the liver and kidney were about 1000-fold higher than that in the pregnancy serum.

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Table I. Polyamine oxidase activities in pregnancy sera and human tissues

	Polyamine oxidase activity (nmol/ml or gm wet weight/60 min)				
Sample	Our study	Results of Morgan's group ^{3-5,8}			
Pregnancy sera					
Early stage	0.20 ± 0.09 $(n = 8)$	1.71			
Middle stage	0.19 ± 0.14 $(n = 5)$	5.09			
Late stage	0.24 ± 0.12 $(n = 16)$	9.20			
Retroplacental sera	$0.63 \pm 0.06*$ $(n = 5)$	292			
Nonpregnancy sera	0.30 ± 0.06 (n = 8)	0.10			
Human liver	239 ± 118 $(n = 4)$				
Human kidney	258 ± 152 $(n = 4)$				
Human brain	65.2 ± 5.0 $(n = 4)$				

Values are means \pm SD. Numbers of samples are in parentheses.

*p < 0.01, by t test, compared with value for nonpregnancy sera.

Comment

Polyamines, such as putrescine, spermidine, spermine, and their acetylated derivatives, are widely accepted to be closely associated with active cell growth, such as in neoplasms and pregnancy. The polyamine levels in plasma were reported to be significantly elevated during late stages of pregnancy.7 Morgan's group^{3-5, 8} reported high levels of activity of polyamine oxidase, an enzyme that is responsible for the metabolism of polyamines and their acetyl derivatives in pregnancy serum (Table I). In our study we used a newly established assay method⁶ for the measurement of polyamine oxidase in human pregnancy serum. It is highly specific, sensitive, and not interfered with by diamine oxidase activity that is very high in pregnancy serum.9-11 As a result, polyamine oxidase activity in pregnancy serum was very low and did not increase during gestation, which is discrepant from the findings of Morgan's group, who used radioactive spermine as substrate (Table I). Their high levels of activity are probably due to contamination by diamine oxidase, which is abundant in pregnancy serum, because spermine is oxidized to some extent by diamine oxidase12, 18 and the resulting radioactive aldehyde product probably is not separated from spermidine, the reaction product of polyamine oxidase for scintillation counting, under their conditions. In their method of polyamine oxidase assay the incubation mixture should have contained 1 mmol/L semicarbazide or aminoguanidine to suppress diamine oxidase activity. However, the sensitivity of their method, after the addition of a carbonyl reagent, will become much lower than that of our method, because polyamine oxidase activity toward spermine is usually much lower than that toward N¹-acetylspermine.²

We have observed significantly higher levels of polyamine oxidase activity in retroplacental serum, although it is almost negligible as compared with that in tissues (Table I). This is probably due to contamination of retroplacental serum by small membrane fragments derived from placental or uterine tissues containing polyamine oxidase during its sampling; such contamination is likely because polyamine oxidase is a ubiquitous membrane-bound enzyme in mammalian tissues.^{2, 14} The polyamine oxidase activity in retroplacental serum reported by Morgan's group is 463-fold higher than that in our study (Table I); their results obviously reflect diamine oxidase activity.

Polyamine oxidase (Enzyme Commission 1.4.3.4) is distinct from diamine oxidase (Enzyme Commission 1.4.3.6), although both enzymes show activities toward spermine and spermidine. Confusion of them is misleading and should be avoided by the use of a specific substrate and/or a specific inhibitor.

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Amnioinfusion increases amniotic pressure in pregnant sheep but does not alter fetal acid-base status

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To investigate the recent suggestion that fetal hypoxemia and acidemia in polyhydramnios are secondary to raised amniotic pressure, 5 to 15 L of normal saline solution was infused intraamniotically at 100 ml/min in seven ewes. Amniotic pressure increased linearly by 1.0 \pm 0.013 (mean \pm SEM) mm Hg per liter infused. Infusion of 15 L produced a mean rise in amniotic pressure of 15.2 \pm 1.2 mm Hg (p < 0.001) but no significant change in fetal pH, Pco₂, Po₂, fetal heart rate, or mean arterial pressure. A similar rise in amniotic pressure and lack of change in blood gas values occurred in two control ewes infused intraperitoneally. Rupture of the membranes occurred in two of three amnioinfusions of 15 L. We conclude that acute amnioinfusion raises amniotic pressure in sheep but does not alter fetal blood gas status. (AM J OBSTET GYNECOL 1991;165:1459-63.)

Key words: Polyhydramnios, amniotic pressure, fetal blood gases, fetal acid-base status

The excess perinatal morbidity and mortality associated with polyhydramnios are attributable in part to an increased incidence of preterm delivery because of preterm labor and ruptured membranes. Other reasons for adverse perinatal outcome in polyhydramnios include congenital malformations and fetal compromise.

Recent studies in women undergoing invasive procedures have recorded raised amniotic pressures in polyhydramnios, which return toward normal with drainage of amniotic fluid.1-3 It has been suggested that the complications of polyhydramnios may be mediated through raised amniotic pressure.8 In regard to fetal compromise, we have reported significant inverse correlations between amniotic pressure and both fetal pH and Po₂ in 14 pregnancies with polyhydramnios investigated by fetal blood sampling.8 Case reports suggest that fetal condition in polyhydramnios can be ameliorated by reducing high amniotic pressure by drainage of fluid; Tabor and Maier4 noted the disappearance of fetal heart rate (FHR) decelerations as amniotic pressure fell from 50 to 10 mm Hg, whereas we observed that restoration of pressure to within normal limits was accompanied by correction of fetal acidemia and hypoxemia.³ Several authors have thus speculated that raised amniotic pressure in polyhydramnios may impair uteroplacental perfusion.^{1, 3, 4} On the other hand, clinical studies of fetal condition in severe polyhydramnios may be confounded by underlying etiologic conditions such as hydrops and fetofetal transfusion.⁵ Indeed, only a third of the variance in fetal blood gas status in that study³ could be attributed to amniotic pressure.

The aim of this study was to determine, in the sheep, the effect on fetal blood gas status of increasing amniotic pressure by fluid infusion.

Material and methods

Surgical procedure. Chronically instrumented fetal sheep were prepared as previously described6 with standard procedures approved by the host institution and in accordance with United Kingdom legislation. Briefly, anesthesia in nine cross-bred ewes at 116 to 126 days' gestation was induced with thiopental sodium (1 gm given intravenously) and maintained with halothane (1.5% to 2.0% in oxygen). One horn of the gravid uterus was exposed at laparotomy, and the fetal head was delivered through a uterine incision. Catheters were implanted in a fetal carotid artery and the amniotic cavity. An additional polyvinyl catheter of 2 mm inner diameter was implanted for fluid infusion in the amniotic cavity in seven ewes and in the maternal peritoneal cavity in two ewes, that served as controls. The fetus was replaced, the uterus was closed in two layers, and all catheters were exteriorized through the ewe's right flank. At least 5 days was allowed for postoperative

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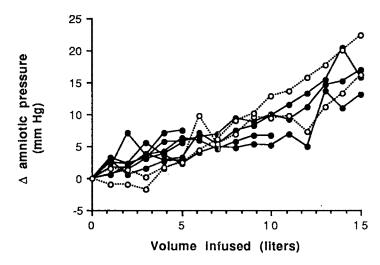


Fig. 1. Change in amniotic pressure with infusion of normal saline solution into amniotic cavity (n = 7, closed circles) or maternal peritoneal cavity (n = 2, open circles).

Table I. Details of linear relationships between volume infused and rise in amniotic pressure

Ewe	Туре	Site	Volume infused (L)	Correlation coefficient	Significance	Slope (mean ± SEM)
A	Twin	Amniotic	5	0.54	p = 0.018	0.60 ± 0.16
В	Singleton	Amniotic	5	0.83	p = 0.003	1.69 ± 0.27
C	Singleton	Amniotic	5	0.96	p < 0.001	1.04 ± 0.07
D	Singleton	Amniotic	10	0.88	p < 0.001	0.79 ± 0.06
E	Twin	Amniotic	15	0.98	p < 0.001	1.05 ± 0.03
F	Singleton	Amniotic.	15	0.96	p < 0.001	1.10 ± 0.05
G	Twin	Amniotic	15	0.84	p < 0.001	0.74 ± 0.06
Н	Twin	Peritoneal	15	0.91	p < 0.001	0.90 ± 0.07
I	Twin	Peritoneal	15	0.96	p < 0.001	1.28 ± 0.06

recovery, during which antibiotics were administered to ewe and fetus.

Experiments. Experiments were performed between 121 and 133 days' gestation in the presence of normal fetal pH and blood gas values (mean ± SEM: pH, 7.33 ± 0.01 ; PCO₂, 38 ± 3 mm Hg; PO₂, 22 ± 1.3 mm Hg). Normal saline solution (0.9% sodium chloride) warmed to 38° to 39° C was infused intraamniotically or intraperitoneally at 100 ml/min. Pressures were measured with standard biomedical transducers and recorded on a polygraph run at 0.25 mm/sec. Arterial samples for blood gas analysis were drawn at 10 minute intervals. Amniotic fluid volume at this gestation approximates 1 L7; therefore intraamniotic infusion of 5 L was estimated to increase amniotic fluid volume by >500%. However, in the absence of change in fetal pH and blood gases or maternal disturbance with intraamniotic infusion of this volume, a further 5 L was infused in one ewe and a further 10 L in three ewes. The maternal flank was examined after each liter for evidence of leakage around the catheters and the perineum was inspected for signs of rupture of the membranes. The volume infused intraperitoneally in control fetuses was 15 L. One hour after the infusion, animals were killed with pentobarbital (4 g given intravenously),

and the gross distribution of infused fluid was confirmed at necropsy. There was no evidence of intraperitoneal leakage in the seven ewes that underwent intraamniotic infusion.

Analysis. Mean amniotic pressure was calculated after every liter from point estimates made every 10 seconds over 2 minutes. In the presence of a contraction, the point estimate was obtained after the next return to baseline amniotic pressure. FHR and mean arterial pressure were similarly recorded in five fetuses. Two-way analysis of variance was used over 5 and 15 L to analyze changes in the various parameters $(\Delta = Postinfusion value - Preinfusion value)$, after their distributions were confirmed as normal by histograms. The nonparametric Wilcoxon test was used for testing the significance of overall changes (postinfusion – preinfusion) in animals undergoing infusions of varying volumes. Linear regression analysis was used only after demonstration of lack of residual relationship with the least-squares method.

Results

Amniotic pressure increased with all seven intraamniotic and two maternal intraperitoneal infusions, as shown in Fig. 1. The slopes of the individual regression

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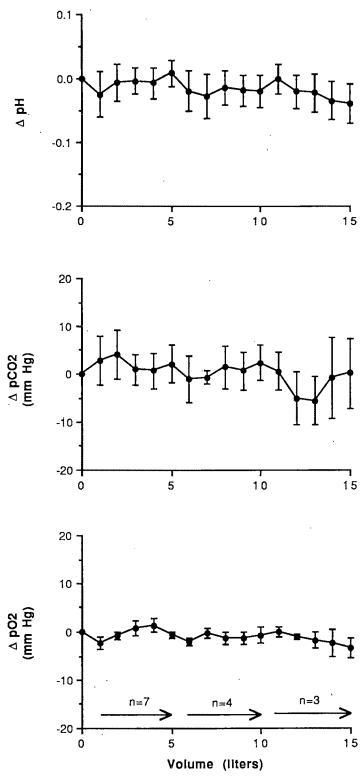


Fig. 2. Mean change in fetal blood gas values with intraamniotic infusion.

equations are shown in Table I. The mean slope in the intraamniotic infusion group (1.00 \pm 0.13) was similar to that in the intraperitoneal infusion group (1.09 ± 0.19) . Although in the intraamniotic infusion group the mean slope was steeper in singleton than in twin pregnancies (1.15 \pm 0.19 and 0.80 \pm 0.13, respectively), this difference was not significant (p = 0.21). In intraamniotic infusions, the slopes were similar in those infused to 5 L compared with those infused to 15 L (1.11 \pm 0.32 and 0.96 \pm 0.11, respectively). There was a significant linear relationship in the intraamniotic infusion group between mean Δ amniotic pressure calculated after each liter, and the volume infused (r=0.96, y=0.96x, p<0.001). Rupture of the membranes was noted at 15 L in two of the three undergoing intraamniotic infusion of this volume, leading to falls in pressure over the next hour of 9.8 and 6.1 mm Hg (58% and 39%, respectively, of the overall Δ amniotic pressure with infusion). The corresponding fall after infusion in the pregnancy with intact membranes infused to 15 L was 3.7 mm Hg (16% of overall Δ).

Intraamniotic infusion over 50 minutes of the first 5 L increased mean amniotic pressure by 4.8 ± 0.7 (SEM) mm Hg (F ratio = 12.6; p < 0.001) but did not produce any significant change in fetal pH $(\Delta = 0.01 \pm 0.02; F = 0.7), Pco_2 (\Delta = 2.2 \pm 3.9 \text{ mm})$ Hg; F = 0.4) or Po_2 ($\Delta = -0.6 \pm 0.6$ mm Hg; F = 1.7). Infusion of a further 5 L in one ewe similarly failed to produce any significant change in fetal blood gases; therefore a total of 15 L was next infused intraamniotically in three ewes, producing a mean rise in amniotic pressure of 15.2 ± 1.2 mm Hg (F ratio = 20.7; p < 0.001) but no significant change in fetal pH ($\Delta = -0.04 \pm 0.03$; F = 0.7) fetal Pco₂ $(\Delta = -0.2 \pm 7.3 \text{ mm Hg; } F = 0.4), \text{ or } Po_2 (\Delta =$ 3.3 ± 2.0 mm Hg, F = 1.7). There was similarly no change in the intraperitoneal infusion group $(\Delta pH = 0.002 \pm 0.002, F = 2.0; \Delta Pco_2 = 0.8 \pm 5.5,$ F = 0.5; $\Delta Po_2 = 0.5 \pm 0.5$, F = 1.2). The median overall change in pH in seven intraamniotic infusions was 0.00 (Wilcoxon p = 0.7); the change was 7.9 mm Hg (p = 0.3) in PcO₂ and -1 mm Hg (p = 0.2) in PO2. Mean values for fetal pH and blood gases after each intraamniotic liter are shown in Fig. 2.

There was no significant change in FHR or mean arterial pressure (F ratio 2.0 and 1.8, respectively) with intraamniotic infusion of 10 L. Furthermore, infusion of another 5 L in two of these fetuses produced no subsequent change in FHR or mean arterial pressure (F ratio 1.5 and 1.1, respectively). Intraperitoneal infusion similarly had no effect on FHR or mean arterial pressure (F ratio 0.4 and 1.0, respectively).

Comment

The lack of effect on fetal blood gas status of raising amniotic pressure over the acute period of this study does not support the recent hypothesis that raised amniotic pressure compromises fetal condition.^{3,4} Human studies suggested that this association of fetal hypoxia with elevated amniotic pressure might be mediated through a reduction in uteroplacental perfusion.^{3,4} Animal studies indicate that uteroplacental perfusion needs to be reduced by ≥50% before any effect of fetal blood gas status is demonstrated.^{8,9} Another mecha-

nism might be umbilical venous compression; again experiments in fetal lambs suggest that acute reductions in umbilical venous flow of <50% produce only a slight reduction in Po₂. Our study only elevated amniotic pressure acutely, and it remains possible that chronic elevations in pressure may affect fetal blood gas status. Nevertheless, the rapid improvement in fetal condition with drainage of amniotic fluid in human polyhydramnios suggests a more immediate effect. A

Although the Δ amniotic pressure after 15 L (15 mm Hg) was at the lower end of the pressure found in human pregnancies complicated by gross polyhydramnios (about 15 to 30 mm Hg),2,3 rupture of the membranes, widely assumed to be a pressure effect, occurred in two of three ewes infused intraamniotically with this volume. Amniotic volume was not measured in this study, in view of the difficulties of ensuring adequate mixing of tracers or dyes in an enlarged and acutely expanding amniotic cavity. In the absence of signs of intraperitoneal leakage, it seems highly likely that some of the infused fluid was absorped into the maternal circulation, either directly or by the fetal circulation.11-13 Consistent with this is our anecdotal observation of maternal polyuria during larger volume infusions. Nevertheless, the aim of this study was to increase pressure rather than volume, and the net effect of any absorption that may have occurred did not prevent the continued rise in pressure with increasing volume or the uterine distension observed at necropsy.

The accuracy of estimates of amniotic fluid volume has been greatly improved by modern measurement techniques involving multiple samplings with different radioactive labeled tracers. The studies of Tomoda et al.7,11 indicate that the normal volume in late-gestation ewes is approximately 1 L. They demonstrated that amniotic fluid volume increases by 110% immediately after a 20-minute intraamniotic infusion of 1 L of normal saline solution but returns to normal 24 hours later.11 Initially we infused 5 L so that the estimated fivefold increase in amniotic volume would be analogous to the human pregnancies complicated by gross polyhydramnios.14 After no effect was achieved on fetal blood gas status and only a modest rise in amniotic pressure was produced, the volume infused was increased to 10 and then 15 L. Intraamniotic infusion of 15 times normal volume not only increased amniotic pressure substantially but also appeared to achieve excesses of amniotic fluid considerably greater than found in human polyhydramnios.

This study confirms previous reports that amnioinfusion increases amniotic pressure. Our group has shown that amnioinfusion of 55 to 500 ml increases amniotic pressure by a mean of 4.7 mm Hg in women with midtrimester oligohydramnios.³ Posner et al.¹⁵ recently showed similar rises with intrapartum amnioin-

fusion at term. In sheep, Gilbert and Brace¹⁸ noted an increase in amniotic pressure of 1 to 2 mm Hg after infusing 1.5 L of water, suggesting an ovine uterine compliance of 1 mm Hg per liter of water. The slope of the linear relationship we observed between amniotic pressure and volume infused not only confirms this suggestion but extends it to infusions of 15 L of normal saline solution. The rise of only 15 mm Hg in amniotic pressure after amnioinfusion of 15 L together with the finding that intraperitoneal compliance is similar to amniotic compliance suggests that the ovine uterus may be relatively more distensible than the human uterus. Indeed, during an epidemic of ovine hydramnios, amniotic volumes of 8 to 18 L were recorded in pregnancies of prolonged gestation.¹⁶

Caution must be exercised before extending these findings to human pregnancies complicated by polyhydramnios, in view of considerable interspecies differences. In contrast to the human uterus, the ovine organ is thin-walled and bicornuate. In addition, sheep have a T-shaped allantoic cavity, one arm of which extends into the nonpregnant horn in singleton gestations, and its presence might conceivably dissipate the effects of increasing amniotic pressure.

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The effect of hypothalamic lesions on the length of gestation in fetal sheep

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Twenty-nine sheep fetuses were subject to stereotaxic surgery at 106 to 110 days of gestation. Electrolytic lesions were placed bilaterally in the anterior hypothalamus. Sham-operated controls (n=4) were delivered at 146.3 \pm 4.3 days. Of the fetuses with lesions, two were excluded because histologic confirmation of the lesion was not possible. Those fetuses with bilateral lesions of the suprachiasmatic nuclei (n=4) were delivered at 148 \pm 10 days. In 10 animals with bilateral lesions of the paraventricular nuclei, delivery was significantly (p<0.0001) prolonged to at least 165.6 ± 5.1 days. In nine animals with lesions not involving the endocrine hypothalamus, delivery was at 148.1 ± 4.3 days. All animals that were delivered after 157 days (n=9) had lesions including the paraventricular nuclei bilaterally (p<0.01). The adrenal glands of fetuses with prolonged gestation were normal in weight and light microscopic appearance. These observations demonstrate that fetal neural pathways involving the paraventricular nuclei are essential for parturition in the sheep. However, fetal adrenal growth can continue without such influences. (AM J OBSTET GYNECOL 1991;165:1464-8.)

Key words: Parturition, fetal sheep, hypothalamus, adrenal

The early studies of Liggins et al.1.2 clearly established the role of the adrenocorticotropic axis in determining the length of gestation in sheep. Fetuses subject to hypophysectomy or pituitary stalk section showed prolonged gestation, whereas fetuses treated with glucocorticoids delivered prematurely. Further studies showed that delivery could also be prevented by adrenalectomy. It is now known that the rise in fetal circulating glucocorticoids late in gestation in sheep³ induces the prostaglandin cascade which leads to parturition. However, while such observations clearly show that a fetal pituitary-dependent rise in glucocorticoids is essential for the onset of parturition in sheep,4 the involvement in the fetal brain in this process has been debated.5 It may be that the fetal brain initiates the process by signaling an increase in corticotropin release. Alternatively, the release of pituitary corticotropin may be driven by extraneural influences. A further possibility is that the presence of the fetal pituitary is merely permissive to other influences such as prostaglandins acting directly on the fetal adrenal gland.5

We report the effect of anatomic lesions in two relevant areas of the fetal sheep brain on the length of gestation. Hypothalamic lesions have been induced either in the suprachiasmatic nuclei, which are the major

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Reprint requests: Prof. P.D. Gluckman, Department of Paediatrics, University of Auckland, Private Bag, Auckland, New Zealand. 6/1/30436 site of organization of circadian rhythmicity in the postnatal brain,⁶ or in the paraventricular nuclei, which are the presumed sites of cell bodies containing corticotropin-releasing factor.^{7,8} We report that bilateral lesions of the paraventricular nuclei, but not of the suprachiasmatic nuclei, induce a prolongation of gestation.

Methods

Coopworth ewes mated to Dorset rams were used in these studies. The date of mating at Ruakura Animal Research Station was carefully recorded by daily inspection of a ram carrying a crayon harness. Estrus had been synchronized by progesterone sponge withdrawal, and the ewe was removed from the ram once marking was confirmed. The day of marking was termed day 0. Pregnancy was confirmed by ultrasonography at approximately 60 days of gestation. Only singleton pregnancies were used. At 106 to 110 days of gestation, under sterile conditions, the fetal head was exposed at hysterotomy and placed in a stereotaxic frame as previously described.9, 10 Bilateral stereotaxic lesions were inserted either at coordinates anteroposterior 26.0 mm, vertical 6.1 mm, and lateral 0.6 mm relative to stereotaxic zero for lesioning of the suprachiasmatic nuclei or at anteroposterior 24.5 mm, vertical 7.4 mm, and lateral 0.4 mm, which were the coordinates for lesioning of the paraventricular nuclei. A monopolar electrode was used, and the current passed was generally 6 to 10 mA for a period of 30 to 45 seconds. In some fetuses the current was passed for 60 to 90 seconds to induce a larger lesion. The electrode was inserted bilaterally in the coronal plane at an angle of 20 degrees to the vertical to avoid damage to the sagittal sinus. The skull was repaired with cyanoacrylate glue, the scalp replaced, the fetus returned to the uterus, and 80 mg gentamicin was instilled in the uterus, which was closed. No instrument was placed in the uterus, to avoid any effect of exteriorized instrumentation on gestational length.

After surgery the ewes were housed indoors and fed hay and concentrates at will. The lighting cycle was kept constant at 12 hours light and 12 hours dark, and the ewes were inspected daily. If the ewes were in labor, as defined by the presence of blood or membranes in the vagina and obvious contractions, the ewe was anesthetized, the fetus was removed, and its brain perfused. Some fetuses were born alive at vaginal delivery, and these lambs were killed by barbiturate overdosage. If the fetus had not delivered by at least 157 days after conception (which is mean \pm 3 SD gestational length for animals of this flock), the ewe was similarly anesthetized and the fetal brain obtained between 160 and 172 days.

After fixation of the fetal brain, the anatomic site of lesion was confirmed histologically.9, 10 Briefly, the brain was perfused in situ with 100% saline solution, followed by formalin in saline solution as described previously.9, 10 The diencephalic block was embedded in a gelatin-egg albumin mixture, and 50 µm sections were cut and stained by the Nissl method. Particular attention was focused on whether the paraventricular or suprachiasmatic nuclei had been destroyed. The brains were examined without reference to the experimental protocol or knowledge of gestational length. The integrity of the pituitary stalk and the median eminence of the hypothalamus also was inspected.

In addition to the fetuses with lesions, four fetuses were subjected to sham operation in which electrodes were inserted into the general vicinity of the anterior hypothalamus, no current was passed, and the electrodes were withdrawn.

In the latter part of the study, as a result of the observation that some fetuses had prolonged gestations, bilateral adrenal weights were obtained. These had not been recorded for the initial 14 fetuses. These glands were fixed in formalin, sectioned, stained with hematoxylin and eosin, and examined as described previously.11

The lengths of gestation were compared by analysis of variance. Where animals were killed before labor, the age used for gestational length was the age at death.

The protocols were approved by the Animal Ethical Committee of the University of Auckland, and the animals were cared for in accordance with the local institutional guidelines.

Results

Twenty-nine fetuses were operated on, and 4 of them were sham-operated controls, 9 had lesions with coordinates aimed at the suprachiasmatic nuclei, and 16 had lesions aimed at the paraventricular nuclei. Two fetuses were excluded from the analysis; one that aborted at 139 days and one that was delivered stillborn at approximately 147 days; because the neural tissues had undergone autolysis and were not suitable for histologic analysis.

The 27 suitable fetuses were assigned to five groups according to the histologic findings (Fig. 1): those with bilateral lesions of the paraventricular nuclei not involving the suprachiasmatic nuclei or median eminence (n = 4), those with bilateral lesions of the suprachiasmatic nuclei not involving the paraventricular nuclei or median eminence (n = 4), extensive lesions involving bilaterally both the paraventricular nuclei and the suprachiasmatic nuclei (n = 6), those with misplaced lesions of the diencephalon not involving the paraventricular nuclei, suprachiasmatic nuclei, or median eminence (n = 9), and the sham-operated fetuses (n = 4). Of those with misplaced lesions, 5 had rostrally placed lesions in the septal region, and 4 had caudally or dorsally placed lesions lying outside the hypothalamus.

The sham-lesioned fetuses were delivered at 146.3 ± 4.3 (SD) days (range, 142 to 152 days) (Table I). Of the 9 fetuses with misplaced lesions, 1 aborted at 123 days. The remaining 8 were delivered at 148.1 ± 4.3 days (range, 141 to 153 days). Three of 4 fetuses with bilateral lesions of the suprachiasmatic nuclei were delivered at 151 ± 1 days, and 1 was delivered prematurely at 133 days. Of the 4 fetuses with bilateral lesions of the paraventricular nuclei without involvement of the suprachiasmatic nuclei or median eminence, three were killed in utero at 166 to 172 days and the fourth entered labor at 162 days. Of 6 fetuses with more extensive lesions involving both the paraventricular and suprachiasmatic nuclei bilaterally, 2 also had destruction of the median eminence and were killed at 165 and 171 days. The other 4 had an intact median eminence. One was delivered at 156 days and I at 168 days; the other 2 were killed at 170 and 172 days. Thus the mean age at delivery of the 8 animals with lesions of the paraventricular nuclei but with an intact median eminence was $>165 \pm 5.3$ days. There was no overlap between gestational length in animals with lesions of the paraventricular nuclei and the sham or misplaced lesion groups (p < 0.001). By analysis of variance the effect of paraventricular nuclei lesioning on gestational length was significant (p < 0.01); the effect of suprachiasmatic nuclei lesioning was not (p > 0.2). In total, 9 animals failed to deliver within 3 SDs of term gestation (<157 days), and all 9 had lesions of the paraventricular nuclei (Fisher's exact test, p < 0.01).

The adrenal glands were weighed in 4 fetuses with prolonged gestation and in 6 other fetuses. In those fetuses with parturition delayed until >157 days, the

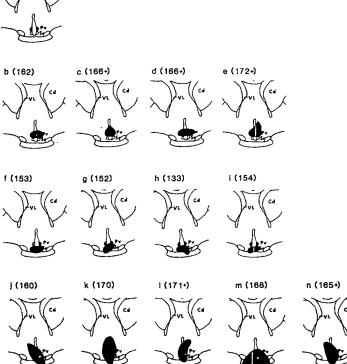


Fig. 1. Placement of lesions in fetuses with lesions in endocrine hypothalamus. Part a Represents normal anatomy at level of optic chiasm. Parts b to e are fetuses with lesions restricted to paraventricular nuclei and surrounding areas; extent of lesion is indicated by solid blocking. Gestational age at delivery is indicated in parentheses. Parts f to i are lesions restricted to suprachiasmatic nuclei and surrounding areas. Parts j to o are larger lesions including both the suprachiasmatic nuclei and paraventricular nuclei. SC, Suprachiasmatic nucleus; PV, paraventricular nucleus; VL, lateral ventricle; Cd, caudate nucleus.

Table I. Results in 27 fetuses assigned to five groups

	No.	. Gestational length (days)	Mean ± SD
Sham	4	142, 144, 152, 147	146.3 ± 4.3
Lesions outside endocrine hypothalamus	9	123, 144, 153, 148, 153, 141, 146, 150	148.1 ± 4.3*
Bilateral lesions of Supra- chiasmatic nuclei	4	133, 152, 153, 154	148 ± 10
Bilateral lesions of para- ventricular nuclei	4	162, 166,† 166,† 172†	$166.5 \pm 4.1 \ddagger $
Bilateral lesions of para- ventricular nuclei ex- tending to suprachias- matic	6	156, 160, 168, 165,† 171,† 170†	165.0 ± 5.9‡\$

^{*}Excluding one fetus that aborted at 123 days.

adrenal weight was 665 ± 227 mg. The average bilateral weight in fetuses delivered at term was 550 ± 261 mg, which is comparable to that previously reported for this flock." The light microscopic architecture of

the glands from postmature fetuses was not distinguishable from that of term fetuses.¹¹ In particular, there was proportionate development of each of the gland components (Fig. 2). The volume densities as a

o (156)

[†]Killed at age indicated.

 $[\]ddagger p < 0.01$, compared with sham-operated animals.

^{\$}These ages are underestimates in that they include fetuses killed before delivery.



Fig. 2. Photomicrograph of adrenal gland from fetus (k, Fig. 1) with bilateral lesions of paraventricular and suprachiasmatic nuclei; animal was killed at 166 days of gestation. Total adrenal weight was 560 gm; fetal weight was 5.22 kg.

percentage of total gland volume11 for each zone of the gland was: zona glomerulosa, 14% ± 4% for prolonged gestation and $12\% \pm 5\%$ for term gestation; zona fasciculata, $44\% \pm 2.5\%$ for prolonged gestation and $43\% \pm 4\%$ for term gestation; medulla, $32\% \pm 2\%$ for prolonged gestation and 30% \pm 5% for term gestation.

Comment

These observations demonstrate that integrity of the paraventricular nuclei or neighboring areas is essential for the normal initiation of the parturition cascade in sheep. The paraventricular nuclei are the major sites of cell bodies of hypothalamic corticotropin-releasing factor neurons.7.8 It has been previously reported that lesions similar to those in the present study are associated with a reduction in the corticotropin response to hypotension or hypoxia in the fetal sheep. 12 Thus there is compelling evidence that the paraventricular nuclei are involved in the control of corticotropin release in fetal sheep. However, previous observations merely demonstrate that the paraventricular nuclei are essential for stress-related corticotropin release in utero. Our data indirectly suggest that the paraventricular nuclei are necessary for the normal preparturient rise in corticotropin levels3 and that if these paraventricular nuclei are lesioned, corticotropin levels cannot rise sufficiently for the prepartum glucocorticoid surge to occur.

In spite of the presumed absence of a preparturient rise in corticotropin, there was no evidence of adrenal atrophy. In contrast, adrenal atrophy is reported after fetal hypophysectomy.13 It is possible that factors other than corticotropin-releasing factor of paraventricular origin are sufficient for adrenal growth by maintaining

nasal corticotropin secretion. In earlier study of fetuses after paraventricular nuclei lesions were found, basal corticotropin levels were normal although hypoxia- and hypotension-induced corticotropin release was abolished.12 It has been suggested that vasopressin,14 which is secreted by cell bodies in both the paraventricular nuclei and supraoptic nuclei and is found in these nuclei in late-gestation fetal lambs,15 is a stimulus to corticotropin release in utero. However, only the latter could be a source of vasopressin for adrenal maintenance in the fetus with paraventricular nuclei lesions. The possible role of corticotropin-releasing factor of placental origin¹⁶ cannot be discounted. However, whereas these extraparaventricular factors can maintain fetal adrenal growth either directly or indirectly, they alone do not allow the normal parturition cascade to occur.

The signal for the prepartum rise in paraventricular nuclei-induced corticotropin release in late gestation in the fetal sheep is not known. Among the hypotheses that have been proposed is that the rise is determined by an intrinsic fetal circadian oscillator. A number of fetal circadian rhythms have been described; these include rhythms of fetal breathing and electroencephalographic activity,17 fetal prolactin,18 and cortisol release.19 The latter may well be secondary to changes in maternal cortisol production; however, the basis of the fetal diurnal rhythms in prolactin and electrophysiologic activity is not known. It has been reported that there is a diurnal rhythm in cerebrospinal fluid arginine vasopressin concentrations.20 As arginine vasopressin in the cerebrospinal fluid originates in the suprachiasmatic nucleus21-23 and not in the magnocellular system,

this rhythm is indirect evidence that suprachiasmatic nuclei do exhibit circadian rhythmicity in fetal sheep. Circadian rhythmicity of metabolic activity has been shown in fetal suprachiasmatic nuclei both in the rhesus monkey24 and in the rat.25 Thus it is not unreasonable to suggest that the fetal suprachiasmatic nuclei show circadian oscillation in fetal sheep. However, our studies clearly show that the suprachiasmatic nuclei do not play a major role in determining the length of gestation in this species. In our studies there were four fetuses with complete destruction of the suprachiasmatic nuclei, and all were delivered before 152 days of gestation. The slight tendency to longer gestation in some of these may be due to the lesions impinging on pathways linking the anterior hypothalamus to the paraventricular nuclei and thus partially affecting corticotropin release.

While the suprachiasmatic nuclei may have no role to play in the timing of the length of gestation, our observations do not preclude the possibility that they play a role in the temporal timing of delivery. It has previously been suggested in the rat that, in addition to a biologic clock that determines the length of gestation, there may be an independent clock that determines when, within a window of gestational length, delivery actually occurs.26 Such a gating system has been used to explain the circadian rhythm in the time of delivery, which is particulary marked in some species. We do not have sufficient data to address this question in this study.

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Lymphocytes stimulate progesterone production by cultured human granulosa luteal cells

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After follicular rupture, massive invasion of blood vessels with neovascularization of the developing corpus luteum takes place, providing many chances for direct contact of luteal cells with resident and migrating immune cells. We studied the effects of peripheral blood lymphocytes on progesterone production by human granulosa luteal cells isolated from women undergoing in vitro fertilization. During 6 days of culture. progesterone production by granulosa luteal cells was significantly increased when they were cultured together with autologous or allogenic peripheral blood lymphocytes. This stimulatory effect was also observed on the addition of medium conditioned with peripheral blood lymphocytes and was synergistic with gonadotropin stimulation. The activity was present in the fraction retained by ultrafiltration with a 30,000 molecular weight cutoff filter and was preserved after heating at 56° C for 30 minutes but disappeared after heating at 70° C for 15 minutes. These findings suggest that lymphocytes infiltrating the corpus luteum during early luteinization can stimulate the function of human granulosa luteal cells through the action of some protein-like humoral factor(s) of higher molecular weight than that of previously identified lymphokines and indicate a possible paracrinologic regulatory role for lymphocytes in ovarian function. (AM J OBSTET GYNECOL 1991;165:1469-74.)

Key words: Human granulosa luteal cell, progesterone production, lymphocyte, cytokine

In recent years a growing body of evidence has accumulated to suggest that the immune system is an additional local regulator of ovarian function. It is well known that immunoincompetent or immunosuppressed animals show numerous reproductive disorders.1-8 Thymic control of ovarian function has clearly been demonstrated by the experimental induction of oophoritis in neonatally thymectomized mice.4.5 Macrophages have been identified in the developing corpus luteum in several species, including man,6-8 and have been suggested to be putative intraovarian regulators. One macrophage-derived cytokine, interleukin-1, has been observed to suppress the functional and morphologic luteinization of cultured murine and porcine granulosa cells.9, 10 Another cytokine, tumor necrosis factor-α, has been reported to inhibit the gonadotropin-dependent differentiation of murine granulosa cells and to cause complex dose-dependent alterations in the elaboration of progesterone and androstenedione. 11-13 On the other hand, lymphocyte secretory products (lymphokines) have been shown to have an effect on steroidogenesis in cultured rat granulosa luteal cells. However, it is unclear whether the function of granulosa luteal cells can be modulated by other immune cells, especially lymphocytes, which should play a central role in the immune response, and whether the stimulatory effect can be observed in an autologous combination occurring inside ruptured follicles in vivo. Accordingly, we examined the effect of lymphocytes of autologous origin and the conditioned medium derived from lymphocytes on progesterone production during the culture of granulosa luteal cells obtained from patients at oocyte retrieval in our in vitro fertilization program. The ovarian stimulation was controlled completely by exogenous gonadotropins that mimic the normal ovulatory cycle. Thus granulosa luteal cell culture for 6 days after oocyte retrieval is considered to be an appropriate in vitro model for studying early luteinization.

Material and methods

Granulosa luteal cells were obtained from patients undergoing superovulation in the in vitro fertilization program at Kyoto University Hospital. They had primary or secondary infertility with fallopian tube occlusion but no endocrinologic disorders. Follicular development was induced with 150 IU human menopausal gonadotropin (Pergonal, Teikoku-zoki, Tokyo) given

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from day 3 of the cycle and with 900 µg gonadotropinreleasing hormone analog (buserelin acetate, Hoechst Japan, Tokyo), provided as a nasal spray, daily from day 1 of the cycle. Follicular maturation was assessed each day by measurement of serum estradiol levels and ultrasonographic scanning. When the leading follicle reached 18 mm in diameter, human menopausal gonadotropin was discontinued and 5000 IU human chorionic gonadotropin (hCG) (hCG Mochida, Mochida Pharmaceutical, Tokyo) was injected 52 hours after the last human menopausal gonadotropin administration. Oocytes were recovered 36 hours after the injection of hCG through the ultrasonographically guided transvaginal route.

Granulosa luteal cells were obtained by using a heparinized buffer (Dulbecco's phosphate-buffered saline solution, Gibco, Grand Island, N.Y.) to wash the follicles so as to enhance the recovery of oocytes. After removal of the oocytes, the granulosa luteal cells were pooled, centrifuged at 200 g for 20 minutes, and resuspended in RPMI 1640 medium (Gibco). Separation of the granulosa luteal cells from red blood cells was achieved with a Ficoll gradient (Lymphocyte Separation Solution, Nakalai Tesque, Kyoto). The viability of the granulosa luteal cells was assessed by trypan blue staining and was usually >90%. The granulosa luteal cells were then centrifuged and resuspended in RPMI 1640 medium containing 10% fetal calf serum and 100 U/ml penicillin (complete medium). The cells were plated at a density of 1 × 10⁵ viable cells per well in 24-multiwell culture plates (Corning Glass Works, Corning, N.Y.) containing 1 ml complete medium and precultured for 24 hours at 37° C under humidified 5% carbon dioxide in air.

Peripheral blood mononuclear cells were isolated from male or female volunteers by means of centrifugation with a Ficoll gradient at 24 hours after oocyte retrieval. Peripheral blood mononuclear cells were resuspended in complete medium and then counted with a hemacytometer. They were then incubated for 1 hour at 37° C in plastic dishes that had been precoated with autologous serum for 15 minutes at 37° C. Nonadherent cells were collected from the dishes and were shown to contain <5% monocytes by immunofluorescence staining by Leu M3 monoclonal antibody (Becton-Dickinson Immunocytometry System, Calif.). These cells were defined as peripheral blood lymphocytes. The adherent cells were removed from the dish by incubation for 15 minutes with ethylenediaminetetraacetate (1:5,000, Gibco) at room temperature followed by gentle scraping with a rubber policeman. They contained >96% Leu M3-positive cells and were defined as monocytes.

Granulosa luteal cells were cultured for 6 days with the medium being replaced every 2 days, and the basal progesterone production was determined. To examine the effect of hCG on progesterone production, hCG was added to the granulosa luteal cell culture in concentrations from 5 to 50,000 mIU/ml. Autologous or allogenic peripheral blood mononuclear cells, peripheral blood lymphocytes, and monocytes prepared as described were added to the precultured granulosa luteal cells at a density of 1×10^4 to 2×10^6 viable cells per well. Each well contained 2 ml complete medium, and cells were added in the presence or absence of hCG (500 mIIU/ml). All cultures were prepared in triplicate, and the number of granulosa luteal cells in each well was counted by the citric acid—crystal violet method at the completion of culture.

Conditioned medium was prepared by culturing autologous or allogenic peripheral blood lymphocytes in complete medium for 6 days and added to granulosa luteal cell cultures. In addition, we examined the effect of conditioned medium after heat treatment (56° C for 30 minutes and 70° C for 15 minutes). Conditioned medium from peripheral blood lymphocytes was also prepared in RPMI 1640 containing 0.5% bovine serum albumin (BSA, Sigma, St. Louis) and subjected to ultrafiltration with a centrifugal microconcentrator (Centricon-30; 30,000 molecular weight cutoff; 2000 g for 30 minutes; Amicon Division, W.R. Grace, Danvers, Mass.). The retained or filtered fractions were added to the granulosa luteal cell cultures.

Spent culture medium was frozen and assayed for progesterone by radioimmunoassay with a commercial kit (progesterone—iodine 125 kit; Sorin Biomedica, Saluggia, Italy). Progesterone production was expressed in micrograms per 10^5 cells per 48 hours or 6 days. All data are presented as the mean \pm SD of triplicate cultures. Student's t test was used for statistical analysis.

Results

Cultured granulosa luteal cells derived from preovulatory follicles after exogenous administration of hCG in vivo secreted large amounts of progesterone during the first 2 days of culture. In the subsequent culture period, from 2 to 6 days, basal progesterone production tended to decrease. Progesterone production by granulosa luteal cells was little enhanced even by the addition of hCG during the first 2 days of culture. However, from 2 to 6 days, the level of progesterone production of granulosa luteal cells could be boosted by doses of 50 to 50,000 mIU/ml hCG (Fig. 1).

When 1×10^5 granulosa luteal cells were cultured with various densities of autologous peripheral blood mononuclear cells, 1×10^4 or 1×10^5 peripheral blood mononuclear cells did not affect progesterone production by granulosa luteal cells in comparison with

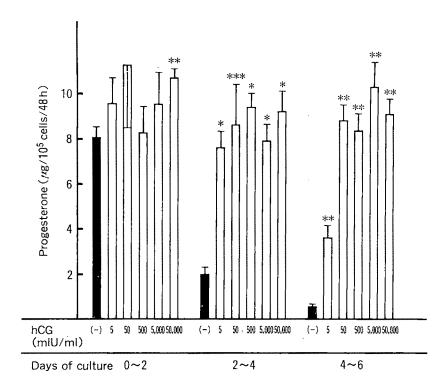


Fig. 1. Basal and hCG-treated progesterone production and effect of hCG on progesterone production by granulosa luteal cells. Five to 50,000 mIU/ml hCG was added to the granulosa luteal cell culture. Asterisk, p < 0.001; two asterisks, p < 0.01; three asterisks, p < 0.05 (all vs control).

the controls. However, 1×10^6 and 2×10^6 autologous peripheral blood mononuclear cells caused a significant increase in progesterone production (p < 0.05, Fig. 2). A similar stimulatory effect of peripheral blood mononuclear cells on progesterone production by granulosa luteal cells was observed when granulosa luteal cells were cultured with 1 × 106 peripheral blood lymphocytes separated from peripheral blood mononuclear cells, but was not observed during culture with 1×10^6 monocytes (Fig. 3). One million fresh human erythrocytes cultured with granulosa luteal cells failed to stimulate progesterone production, indicating that the stimulation of granulosa luteal cells was not due to simple cell-cell contact (Fig. 3). Progesterone and hCG were not detectable in the culture medium when peripheral blood lymphocytes were cultured alone (data not shown).

Granulosa luteal cells were cultured with 1×10^6 autologous peripheral blood mononuclear cells in the presence or absence of hCG (500 mIU/ml). An additive effect of peripheral blood mononuclear cells and progesterone production was also observed in granulosa luteal cells cultured with hCG (Fig. 4). Progesterone was not detectable in the medium of peripheral blood mononuclear cells cultured with hCG (data not shown).

Conditioned medium derived from peripheral blood lymphocytes and added to granulosa luteal cell culture had a significant stimulatory effect on progesterone

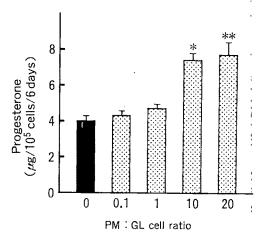


Fig. 2. Effect of autologous peripheral blood mononuclear cells (*PM*) on progesterone production when cultured with granulosa luteal (*GL*) cells. Peripheral blood mononuclear cells (1×10^4 to 2×10^6 cells) were added to 1×10^5 granulosa luteal cells, so ratio of peripheral blood mononuclear cells to granulosa luteal cells ranged from 0.1 to 20. *Asterisk*, p < 0.01; two asterisks, p < 0.05 (all vs control).

production in a dose-dependent manner. There was no difference in the stimulatory effect between the conditioned medium derived from autologous and allogenic peripheral blood lymphocytes (Fig. 5). Counting of granulosa luteal cells at the completion of culture showed no increase in numbers.

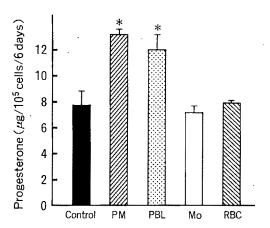


Fig. 3. Effect of autologous peripheral blood mononuclear cells (*PM*), peripheral blood lymphocytes (*PBL*), monocytes (*Mo*), and erythrocytes (*RBC*) on progesterone production by granulosa luteal cells. One million peripheral blood mononuclear cells, peripheral blood lymphocytes, monocytes, or erythrocytes were added to 1×10^5 granulosa luteal cells and cultured for 6 days. *Asterisk*, p < 0.05 (vs control).

Table I. Effect of conditioned medium processed by Centricon-30 on progesterone production by granulosa luteal cells

	Conditioned medium content of culture medium (%, vol/vol)	Progesterone production (% control, mean ± SD)
Retained	8	140.2 ± 18.2
fraction	16	$152.2 \pm 6.1*$
	32	$171.8 \pm 0.4*$
Filtrate	8	90.7 ± 15.2
	16	104.6 ± 7.5
	32	97.0 ± 24.3

^{*}p < 0.05 (vs control).

Table II. Stability of humoral stimulator(s) to heat treatment

Treatment	Conditioned medium content of culture medium (%, vol/vol)	Progesterone production (% control, mean ± SD)
56° C for	20	118.0 ± 7.7
30 min	40	$150.5 \pm 13.3*$
70° C for	20	93.9 ± 15.9
15 min	40	105.4 ± 7.4

^{*}p < 0.05 (vs control).

The retained fraction of conditioned medium derived from peripheral blood lymphocytes after Centricon-30 ultrafiltration significantly stimulated progesterone production by granulosa luteal cells in a dose-

dependent manner, but the filtrate failed to stimulate progesterone production, suggesting that the molecular weight of the stimulatory factor(s) secreted by peripheral blood lymphocytes was more than 30,000 (Table I). Heating of conditioned medium at 56° C for 30 minutes did not alter its stimulatory activity, but the activity was abolished by heating at 70° C for 15 minutes (Table II).

Comment

In this study we demonstrated that secretory products from lymphocytes can stimulate granulosa luteal cell steroidogenesis in vitro in humans. When granulosa luteal cells were cultured together with peripheral blood lymphocytes, progesterone production was increased, and there was no difference in the stimulatory effect of autologous and allogenic peripheral blood lymphocytes. Moreover, the conditioned medium derived from cultured autologous or allogenic peripheral blood lymphocytes also stimulated progesteroné production by granulosa luteal cells in a dose-dependent manner.

Soon after ovulation numerous blood cells rapidly infiltrate the ovarian granulosa cell layer, which is localized in an avascular area defined by the basement membrane before ovulation, and thus come into direct contact with the granulosa cells. Recently, unidentified macrophage factor(s) were found to stimulate progesterone production by murine14 and human15 granulosa luteal cells in vitro. However, the macrophage-derived cytokines interleukin-19, 10 and tumor necrosis factorα¹¹⁻¹³ have been shown to suppress luteinization, implying that there exists a complex regulatory mechanism for ovarian function involving macrophages. In mice, cell-cell contact has been proved to be an essential component of this stimulatory effect of macrophages. 16 On the other hand, the secretory products from concanavalin A-stimulated lymphocytes have been found to stimulate progesterone production by cultured rat granulosa cells.17 In this study we examined the effect of peripheral blood lymphocytes on the luteal function through culture with autologous peripheral blood lymphocytes and granulosa luteal cells as an in vitro model of the early stage of luteinization. Granulosa luteal cells collected from aspirates of mature preovulatory follicles had acquired the capacity for progesterone production by hCG administration. Before starting the experiments, granulosa luteal cells separated on a Ficoll gradient were precultured for 24 hours to allow adhesion to plastic to remove contaminating blood cells. It seems that the level of progesterone production by granulosa luteal cells during the first 48 hours of culture was not increased by the addition of hCG, probably because they had a refractoriness to hCG early in the culture by the high amount of exogenously adminis-

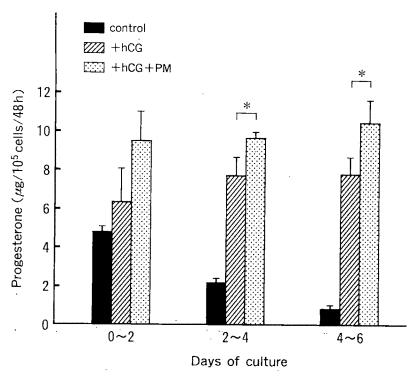


Fig. 4. Effect of peripheral blood mononuclear cells (PM) on progesterone production by hCGtreated granulosa luteal cells. One hundred thousand granulosa luteal cells were cultured with 1×10^6 autologous peripheral blood mononuclear cells in presence or absence of hCG (500 mIU/ml). Asterisk, p < 0.05 (vs culture with hCG only).

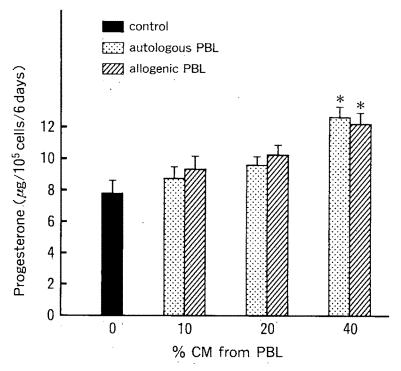


Fig. 5. Effects of conditioned medium (CM) obtained from autologous or allogenic peripheral blood lymphocytes (PBL) on progesterone production by granulosa luteal cells. Conditioned medium obtained from autologous or allogenic peripheral blood lymphocytes was prepared as described in Material and methods and was added to granulosa luteal cell culture. Asterisk, p < 0.05 (vs control).

tered hCG 36 hours before oocyte retrieval.¹⁸ In contrast to the previous report of Halme et al.¹⁵ we could not find a significant stimulatory effect of monocytes or macrophages on granulosa luteal cell progesterone production. In fact, stimulation was observed in cultures of granulosa luteal cells and peripheral blood lymphocytes from which adherent cells (monocytes or macrophages) had been removed.

To determine whether cell-cell contact between granulosa luteal cells and peripheral blood lymphocytes was essential for stimulation of progesterone production, we examined the effect of conditioned medium obtained from peripheral blood lymphocytes on progesterone production by granulosa luteal cells. Conditioned medium derived from peripheral blood lymphocytes cultured for 6 days was found to stimulate progesterone production significantly in a dose-dependent manner, suggesting that the direct contact of peripheral blood lymphocytes and granulosa luteal cells was not required in humans, in contrast with mice.16 This stimulatory effect of the conditioned medium from peripheral blood lymphocytes, which contained no or very few monocytes or macrophages, supported the findings observed in the culture system mentioned above.

The humoral stimulator(s) survived heating at 56° C for 30 minutes and lost its activity after 15 minutes at 70° C; the stimulatory activity was found in the retained fraction after processing with a Centricon-30 and not in the filtrate. These findings suggest that the humoral factor(s) that stimulates progesterone production by granulosa luteal cells seems to be a protein-like substance with a molecular weight of >30,000. The factor(s) cannot be interferon-y, interleukin-1, interleukin-2, and transforming growth factor-β, since all of these cytokines did not stimulate progesterone production by granulosa luteal cells in our granulosa luteal cell culture system (unpublished observations). In the light of these results, the humoral factor(s) responsible for granulosa luteal cell stimulation might be an unidentified cytokine(s) released from lymphocytes.

Peripheral blood lymphocytes had an additive effect on progesterone production by hCG-treated granulosa luteal cells. The stimulatory effect of hCG on progesterone production by granulosa luteal cells reached a plateau when >50 mIU/ml hCG was added. However, an additive effect of culture with peripheral blood lymphyocytes was found when as much as 50 mIU/ml hCG was added to the granulosa luteal cells, suggesting the existence of mechanisms other than hCG for stimulating granulosa luteal cell function.

In this study we demonstrated that autologous peripheral blood lymphocytes can stimulate progesterone production by granulosa luteal cells and that the stimulation is mediated by unidentified product(s) secreted from lymphocytes. Further investigation is needed to

characterize the luteotropic factor(s) secreted by lymphocytes.

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Postbinding insulin resistance around parturition in the isolated rat epitrochlearis muscle

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To determine whether insulin resistance exists in maternal skeletal muscle during pregnancy and how it returns to normal during the postpartum period, 3-O-methyl[¹⁴C]-p-glucose transport and [¹²⁵l]insulin-binding activities were measured in isolated rat epitrochlearis muscle. Maximally insulin-stimulated methylglucose transport activity was decreased on day 20 of pregnancy and on days 1 and 4 post partum; it returned to the nonpregnant level by day 9. The insulin-binding activity did not change significantly throughout pregnancy, increased on days 1 and 4 post partum, and returned to the nonpregnant level by day 9. There was no significant difference in insulin binding or insulin-stimulated methylglucose transport activity between lactating and nonlactating animals. These results suggest that insulin resistance caused by postbinding changes in epitrochlearis muscle develops during late pregnancy and continues at least until day 4 post partum. Lactation does not appear to have a significant effect on insulin resistance. (AM J OBSTET GYNECOL 1991;165:1475-80.)

Key words: Insulin resistance, insulin receptor, glucose transport, pregnancy, lactation

Insulin resistance during pregnancy is a key phenomenon for understanding the mechanism of maternal metabolic adaptation for supplying glucose to the fetus and also for understanding the pathophysiologic characteristics of gestational diabetes mellitus, which may develop if an increase in insulin secretion cannot meet an increase in insulin resistance.

Peripheral insulin resistance during pregnancy has been demonstrated in vivo by a number of experiments, including glucose clamp studies in humans,¹ rats,² and rabbits³ and hind-limb perfusion studies in rats.⁴ However, the results of in vitro studies with isolated tissues have been somewhat conflicting. Some have observed insulin resistance in isolated adipocytes from humans⁵,6 and rats,^{7,8} but others have not observed resistance in isolated adipose tissues° or adipocytes.¹⁰ Although skeletal muscle is the major tissue responsible for peripheral glucose use,² there have been few reports on this subject with the use of isolated skeletal muscle. One article¹¹ could not find any insulin resistance in isolated soleus muscle from pregnant rats.

Our previous experiment⁸ with isolated adipocytes from pregnant rats near term revealed insulin resistance in terms of methylglucose transport activity, and one of the aims of this study is to determine whether a discrepancy exists between the results with isolated adipocytes and those with isolated skeletal muscle. The second purpose of this study is to demonstrate how maternal tissue recovers from its insulin-resistant state after parturition. The last purpose is to determine whether lactation has any effect on insulin resistance.

To answer these questions, we examined changes in methylglucose transport and insulin-binding activities in isolated epitrochlearis muscle strips from rats during pregnancy and the postpartum period, with or without lactation.

Material and methods

Animals. Sprague-Dawley rats 6 weeks old were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and were taken care of in the Institute of Laboratory Animals of Mie University School of Medicine, which approved our experiments. The rats were fed at will in an air-conditioned room at 23° C with 60% humidity, light from 7 AM to 7 PM, and dark from 7 PM to 7 AM. The day on which sperm was found in the vagina was designated as day 0 of pregnancy. The rats were killed at 10 to 11 weeks of age for the experiments on days 10, 15, and 20 of pregnancy and days 1, 4, and 9 post partum, as were virgin rats for the controls. The groups at 4 and 9 days post partum were divided into two groups, lactating and nonlactating. The pups of the nonlactating group were removed from the mother on day 1 post partum.

Isolation of epitrochlearis muscle. The rats were

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decapitated between 11 AM and 3 PM, and a pair of epitrochlearis muscle pieces was removed within 5 minutes. Immediately after the pieces were weighed, they were put into ice-cold medium until the experiment was started. The medium (pH 7.4) was composed of Krebs-Henseleit bicarbonate buffer bubbled with mixed gases (95% oxygen and 5% carbon dioxide), 0.1% bovine serum albumin (fraction V, A-4503, Sigma, St. Louis), and 2 mmol/L sodium pyruvate (Sigma).

3-O-Methylglucose transport activity. Methylglucose transport activity was assessed by a modification of the method described by Wallberg-Henriksson et al. 12 A piece of epitrochlearis muscle was incubated in a polypropylene tube for liquid scintillation (volume, 20 ml) containing 2.0 ml of medium without insulin, and another piece was incubated with 100 nmol/L insulin with shaking back and forth at 110 cycles/min for 30 minutes in an incubator at 37° C. Then each piece was transferred into medium with the same amount of insulin but containing 3-O-methyl[14C]-D-glucose (0.1 μCi; ICN Radiochemicals, Irvine, Calif.) and L-[3H]glucose (0.05 μCi; New England Nuclear, Boston) and incubated under the same conditions. After a 10minute radioactive incubation with insulin or after a 30-minute radioactive incubation without insulin, the piece was recovered and blotted on a sheet of filter paper to remove the medium and frozen in liquid nitrogen. The piece was homogenized by a glass homogenizer with 15% trichloracetic acid, then centrifuged at 1600 g for 10 minutes at 4° C. The radioactivity (disintegrations per minute of carbon 14 and tritium) of 0.8 ml of the supernatant was measured in scintillant (Univer-Gel II, Nakarai Chemicals, Kyoto) by a liquid scintillation counter (Rackbeta 1219, Wallac, Turku).

The amount of methylglucose transported into the cells was calculated by subtracting the nonspecific association of the radioactivity, which was assessed by the radioactivity of L-[³H]glucose. Transport activity was expressed by clearance of methylglucose from the medium per wet weight of the muscle strip (nanoliters per minute per milligram).

Adenosine triphosphate measurements. Adenosine triphosphate levels in the muscle strips just after removal from a rat and after the transport experiment were assessed as follows. A muscle piece was homogenized in 1 ml of cold saline solution and centrifuged at 1600 g for 10 minutes at 4° C. The adenosine triphosphate content of the supernatant was measured by an assay kit (Sigma) that used a bioluminescent method.

Insulin-binding activity. A pair of muscle pieces was incubated at 15° C with shaking at 110 cycles/min in medium containing [A14- 125 I]insulin (0.05 μ Ci; Amersham, Buckinghamshire, U.K.) with or without 10 μ mol/L insulin. The temperature of 15° C may be con-

sidered as unphysiologic. However, at higher temperature increased degradation and internalization of insulin may not reflect true insulin receptor—binding activity.

After 4 hours' incubation, the pieces were transferred to a sheet of filter paper to remove the medium. The radioactivity associated with the muscle pieces was counted in a well-type scintillation counter (ARC 600, Aloka, Tokyo). The specific binding activity was estimated by subtracting the nonspecific binding, which was the amount of [1251]insulin associated with the muscle piece in the presence of 10 µmol/L of insulin. The specific binding activity was expressed as a percent of the total insulin added to the incubation medium per 100 mg wet weight of the muscle.

The degradation of [125 I]insulin during the incubation was estimated by means of trichloracetic precipitation. 13 A portion of the medium after a 4-hour incubation was put into 1 ml of Krebs-Henseleit buffer containing bovine serum albumin, and 1 ml of 15% trichloracetic acid was added. After centrifugation at 1600 g for 10 minutes, the radioactivity of the supernatant and the precipitate was counted.

Serum insulin and blood glucose assays. The serum was separated from the blood within 15 minutes after it was obtained from the animals by decapitation and frozen at -80° C until the hormone assays. Serum insulin was assayed by a radioimmunoassay kit (Dinabot Japan, Tokyo). The blood glucose levels were measured by a glucose analyzer (model 23A, Yellow Spring Instruments, Yellow Springs, Ohio).

Statistics. We used unpaired, one-tailed t tests to determine differences between values in the control and in the pregnant or postpartum rats. The significant changes in insulin binding and glucose transport activities throughout pregnancy and the postpartum period also were confirmed by analysis of variance. Statistical significance was determined at the p < 0.05 level.

Results

Assessment of the experimental procedure. Each of the following assessments was done by five separate experiments. It was determined that the methylglucose transport activity increased linearly up to 15 minutes with 100 nmol/L insulin and up to 45 minutes without insulin. The maximal transport activity was obtained at insulin concentrations of ≥1 nmol/L. The subsequent transport experiments were performed for 10 minutes for assessing maximally insulin-stimulated glucose transport activity and 30 minutes for basal glucose transport activity.

Adenosine triphosphate levels (nanomoles per milligram wet weight) in the muscle pieces just after removal from the rats and after the incubation for methylglucose transport with or without insulin were

Table I. Fundamental characteristics of experimental animals and muscle tissues

	No.	Body weight (gm)	Food intake (gm/day)	Blood glucose (mg/dl)	Serum insulin (µU/ml)	Tissue weight (mg)
Control	60	209.7 ± 2.1	13.6 ± 0.5	72.8 ± 1.2	18.8 ± 1.1	36.1 ± 0.4
Day 10 of pregnancy	30	$219.3 \pm 3.6*$	$16.7 \pm 0.7 \dagger$	71.7 ± 1.8	18.8 ± 1.0	37.0 ± 0.6
Day 15 of pregnancy	30	$238.6 \pm 3.3 \dagger$	$18.1 \pm 0.7 \dagger$	$61.7 \pm 1.1 \dagger$	19.4 ± 1.4	37.2 ± 0.5
Day 20 of pregnancy	28	$285.1 \pm 4.6\dagger$	$20.1 \pm 1.0 \dagger$	$60.2 \pm 1.4 \dagger$	$14.8 \pm 0.6 \dagger$	37.1 ± 0.6
Day 1 post partum	28	$197.3 \pm 4.6 \dagger$	$6.6 \pm 1.2 \dagger$	$67.0 \pm 1.9*$	19.1 ± 1.5	34.8 ± 0.8
Day 4 post partum, lactating	28	213.4 ± 5.0	$23.5 \pm 1.8 \dagger$	70.1 ± 2.3	$14.9 \pm 0.7\dagger$	34.8 ± 0.6
Day 4 post partum, nonlactating	28	209.8 ± 4.3	14.5 ± 0.8	$78.0 \pm 1.8 \dagger$	20.7 ± 1.0	34.9 ± 0.6
Day 9 post partum, lactating	30	$224.7 \pm 3.9 \dagger$	$38.6 \pm 1.6 \dagger$	72.2 ± 1.2	17.0 ± 0.9	36.4 ± 0.7
Day 9 post partum, nonlactating	30	213.5 ± 4.1	$16.3 \pm 0.7\dagger$	$73.5~\pm~1.5$	20.8 ± 0.8	36.5 ± 0.6

Values are mean ± SE.

 2.17 ± 0.09 , 2.13 ± 0.19 , and 2.08 ± 0.09 (mean \pm SE, n=5), respectively, and no significant difference was found.

A time course of the insulin binding activity shows a plateau after 4 hours, and a 4 hour-incubation time was adopted for the following experiments. From the displacement curve of specific [125I]insulin-binding activity by unlabeled insulin, the insulin concentration that gives half-maximal [125I]insulin binding is estimated at about 10 nmol/L (figures not shown).

The percentage of [125] insulin degraded during binding incubation was not significantly different between the experimental groups (15.4% as the mean of all samples).

Fundamental characteristics (Table I). The mean body weight was significantly increased during pregnancy and on day 9 post partum but significantly decreased on day 1 post partum. Food intake was significantly increased during pregnancy and on day 4 post partum with lactation and on day 9 post partum with or without lactation, although it was significantly decreased on day 1 post partum. The wet weight of the muscle pieces was not significantly different among the groups. The blood glucose levels were significantly decreased on days 15 and 20 of pregnancy and on day 1 post partum but increased on day 4 post partum without lactation. The serum insulin levels were significantly decreased on day 20 of pregnancy and on day 4 post partum with lactation.

Methylglucose transport activity (Fig. 1). Maximally insulin-stimulated methylglucose transport activity in the muscle pieces from rats on day 20 of pregnancy and on days 1 and 4 post partum with or without lactation was significantly reduced as compared with that in virgin rats. These changes were also significant by an analysis of variance. There was no significant difference in methylglucose transport activity between lactating and nonlactating groups. Basal glucose transport

activity was slightly reduced in the muscle pieces from rats on day 20 of pregnancy and on days 4 and 9 post partum with lactation.

Insulin-binding activity (Fig. 2). Insulin-binding activity was significantly increased in the muscle pieces from rats on days 1 and 4 post partum with or without lactation as compared with that in virgin rats. These changes also were significant by an analysis of variance. There was no significant difference in insulin-binding activity between lactating and nonlactating rats.

Comment

Skeletal musle is a major tissue that is responsible for peripheral insulin resistance.² However, studies on pregnancy-induced insulin resistance in isolated skeletal muscle are limited. We think isolated epitrochlearis muscle is suitable for this purpose because it is easily removed, thin, and adequately oxygenated.¹⁴ It is more sensitive to insulin than other muscle tissues, such as soleus or extensor digitorum longus, and has a large glucose utilization.¹⁵ The results of our basic experiments on epitrochlearis muscle strips that showed unchanged adenosine triphosphate levels during the transport experiments, a linear uptake of methylglucose in the time course, and saturable insulin-binding activities indicate its applicability in the current study.

Among the characteristics of the animals, a slight but significant decrease in serum insulin concentrations on day 20 of pregnancy seems inconsistent with the previous reports, which demonstrated increased¹⁶ or unchanged^{2, 8} insulin levels in rats during late pregnancy. However, there are also researchers¹⁷ who observed decreased insulin levels in rats near term. The reason for this discrepancy is not clear, but we think one of the causes may be a difference in the time of experiment. Because of the large consumption of glucose by the fetal pups in rats, the blood glucose levels in the mother are considerably lowered in a fasting

^{*}Significant difference as compared with control: p < 0.05.

[†]Significant difference as compared with control: p < 0.01.

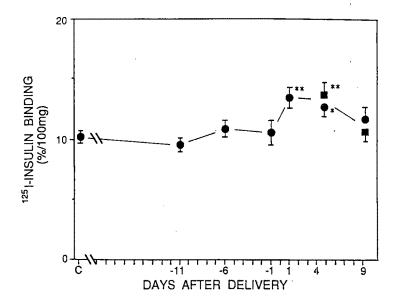


Fig. 1. 3-O-Methylglucose transport activity in isolated epitrochlearis muscle strips from virgin, pregnant, and postpartum rats. Each symbol with bars shows mean \pm SE of 30 separate determinations for control virgin rats and 13 to 15 determinations for other groups. \circ , 3-O-Methylglucose transport activity with 100 nmol/L insulin; \triangle , transport activity without insulin; \square and ∇ , same as above in muscle pieces from rats without lactation. Abscissa shows days after delivery. Delivery occurred on day 21 of pregnancy; therefore, for example, -11 days after delivery is day 10 of pregnancy. C, Control virgin rats. Asterisks demonstrate significant difference from control value by unpaired one-tailed t test (asterisk, p < 0.05; two asterisks, p < 0.01).

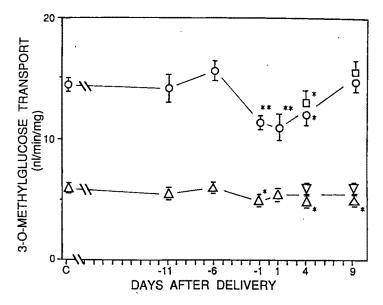


Fig. 2. [1251]insulin-binding activity in isolated epitrochlearis muscle strips from virgin, pregnant, and postpartum rats. Each symbol with bars shows mean \pm SE of 30 separate determinations for control virgin rats and 13 to 15 determinations for other groups. \bullet , [1251]Insulin-binding activity; \blacksquare , [1251]insulin-binding activity in muscle pieces from rats without lactation. Abscissa shows days after delivery. C, Control virgin rats. Asterisks demonstrate significant difference from control value by unpaired one-tailed t test (asterisk, p < 0.05; two asterisks, p < 0.01).

state, namely, during daytime for rats. This markedly low glucose level caused by fetal glucose use may lead to decreased maternal insulin concentration, even if insulin resistance exists in the maternal insulin-sensitive tissue.

The decrease in insulin-stimulated methylglucose transport activity in isolated skeletal muscle on day 20 of pregnancy is in accordance with our previous data in isolated adipocytes. Our findings of in vitro insulin resistance during late pregnancy and the postpartum

period also are in accordance with in vivo findings with the glucose clamp technique.2, 18 The reason for the discrepancy between the data in isolated soleus muscle11 and ours in isolated epitrochlearis muscle is not clear. The effect of insulin on glucose use in soleus and epitrochlearis muscle seems somewhat different according to the previous report¹⁸ on the basis of the glucose clamp technique. Therefore one of the reasons may be a difference in the muscle tissue used. It might be difficult by soleus muscle in vitro to detect a rather small difference in glucose transport activity between pregnancy and nonpregnancy.

Reports on insulin-binding activity in tissues or cells other than skeletal muscle during pregnancy indicate it is increased19 or unchanged,8,20 although a few authors observed a decrease.21 There are relatively few reports on insulin binding after parturition.22 They show unchanged insulin-binding activity at least during the early postpartum period. The insulin-binding activity in isolated skeletal muscle in our experiments was unchanged during pregnancy, was increased during the early postpartum period, and had returned to normal by day 9 post partum. Considering all these reports, changes in insulin-binding activity do not appear to induce insulin resistance around parturition, which means that insulin resistance around parturition is derived from changes in the intracellular process after insulin binds to its receptors.

There is much controversy on insulin resistance during lactation. Some researchers²³ claim that prolactin induces insulin resistance, but others24 do not. Insulin resistance in peripheral tissues in lactating rats has been observed, although the rate of glucose use for the whole body is increased.18 We could not find any significant difference in insulin-stimulated methylglucose transport activity between lactating and nonlactating rats. Our results suggest that lactation does not play a major role in developing insulin resistance during the postpartum period.

In general, low or normal insulin and glucose concentrations may be thought to contradict insulin resistance because typical insulin-resistant diseases such as obesity or insulin-resistant diabetes usually show hyperinsulinemia and hyperglycemia. However, hypoinsulinemia and hypoglycemia are also observed in some insulin-resistant states such as starvation.25 Therefore we think low or normal insulin and glucose concentrations during late pregnancy and the postpartum period in our data do not contradict peripheral insulin resistance. The teleologic meaning of insulin resistance in maternal peripheral tissues during pregnancy is sparing glucose for the fetuses and the maternal brain, which are dependent on glucose as an energy source. The meaning of insulin resistance during the postpartum period seems more complicated. There must be some organ or tissue that requires and consumes glu-

cose spared by the insulin-sensitive tissues. One candidate is the mammary gland, but our data suggest that lactation is not a major determinant of insulin resistance during the postpartum period. It remains to be clarified, but we speculate that insulin resistance in the peripheral insulin-sensitive tissues may be required to spare glucose to restore the depleted glucose storage in the liver or other tissues during the postpartum period.

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The effects of electric field-mediated transfer of nonpermeable molecules of meiosis, fertilization, and early embryo development

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Currently there is no simple method of introducing nonpermeable molecules into oocytes or embryos. A technique that would allow direct access to the cytosol with minimal cell damage would be an extremely useful tool in gamete and embryo research. This study investigates the use of electric field—mediated transfer of nonpermeable molecules into mouse oocytes and embryos. Meiosis II stage oocytes, pronuclear stage zygotes, and two-cell embryos were used to determine optimal voltage settings needed for molecular transfer, viability, and blastocyst transformation in culture. Our highest voltage setting (7.0 kV) yielded molecular transfer, viability, and blastocyst transformation rates of 68%, 73%, and 46%, respectively, in two-cell embryos, whereas our lowest setting (3.75 kV) yielded rates of 28%, 90%, and 47%, respectively. Blastocyst transformation rates for control embryos not exposed to the electric field were significantly higher at 69% (p < 0.01). Melosis, as assessed by germinal vesicle breakdown, was not affected when compared with controls, 78% versus 83%, respectively. We conclude that electric field—mediated transfer of nonpermeable molecules into oocytes and embryos is a simple, relatively atraumatic technique that can be used to study intraoocyte physiologic characteristics and embryo development. (AM J Obster Gynecol 1991;165:1480-6.)

Key words: Electric field—mediated transfer, oocytes, embryos, blastocyst transformation, germinal vesicle breakdown

Gamete and embryo research is hindered by our inability to gain access to the cytosol and nucleus in a simple, atraumatic fashion. Many of the regulatory

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mechanisms of meiosis, fertilization, and embryo development are unknown or poorly understood. A tool that would allow us to monitor biologic effects after introduction of specific proteins, nucleic acids, or antibodies directly into these cells would greatly enhance our ability to understand intracellular physiologic characteristics and control mechanisms. Currently, the method of choice for introducing nonpermeable molecules into oocytes is microinjection. This process, however, is technically difficult, time-consuming, and often traumatic.

1

Recently we have shown that electric field-mediated transfer of enzymes is a simple, efficient method of transferring functional enzymes into human oocytes with minimal cellular damage.1 However, if it is to be used in gamete and embryo research, its effect on meiosis, fertilization, and embryo development must be fully examined. Therefore experiments were designed to answer the following questions:

- 1. Can nonpermeable molecules be transferred into mouse oocytes and embryos without adversely affecting viability, meiotic maturation, fertilization, or blastocyst development?
- 2. When is the optimal time to perform electric field-mediated transfer, i.e., oocyte, zygote or
- 3. What effects on molecular transfer, blastocyst transformation, and viability are seen when the electric field strength is altered?

Material and methods

Oocyte and embryo collection. Four different cell types were investigated: germinal vesicle stage oocytes, meiosis II metaphase stage oocytes, pronuclear stage zygotes, and two-cell embryos. Oocytes and embryos were collected from immature C57BL6-SJLF1 mice (Jackson Labs, Bar Harbor, Me.) Institutional guidelines for care and use of animals were fol-

Superovulation was initiated by intraperitoneal injection of 5 U of pregnant mare's serum gonadotropin (Sigma, St. Louis). Forty-eight hours later 5 U of human chorionic gonadotropin (Sigma) was given intraperitoneally. Fifteen hours later the mice were killed by cervical dislocation, and the oviducts were surgically removed into phosphate-buffered saline solution (Irving Scientific, Irving, Calif.). Cumulus masses were then collected by incising the oviduct with a 25-gauge tuberculin syringe needle. They were placed in a dish of phosphate-buffered saline solution and randomly separated into the various experimental or control groups. All results are collective results from at least seven female mice for each set of results. Cumulus masses containing pronuclear stage zygotes were collected from superovulated immature female mice mated to adult male mice of verified fertility. Meiosis II stage oocytes were collected from superovulated nonmated mice. Two-cell embryos were obtained by incubating pronuclear stage zygotes for 24 hours in Whitten's media containing 3 mg/ml crystalline bovine serum albumin (ICN Biochemicals, Cleveland) in 5% carbon dioxide, balance air, 37° C, and 99% relative humidity.

Germinal vesicle stage oocytes were collected 48 hours after the initial pregnant mare's serum gonadotropin injection. They were obtained by individual follicular puncture of surgically removed ovaries.

Oocytes and zygotes were immediately transferred

to Whitten's media and incubated at 37° C in a 5% carbon dioxide, humidified environment.

Settings used for electric field-mediated transfer. Electric field-mediated transfer was accomplished with a Baekon 2000 gene transfer system (Baekon, Inc., Saratoga, Calif.). Up to 25 oocytes were placed in a Baekon receptacle containing 30 µl of Whitten's media along with the protein or dye to be transferred. The following three settings were used to determine the optimal electric field setting for protein transfer, blastocyst transformation, and viability:

- Setting A. 3.75 kV, 28 pulses, 1.6-second burst time, six cycles, 120-microsecond pulse width
- Setting B. 5.0 kV, 28 pulses, 1.6-second burst time, four cycles, 120-microsecond pulse width
- Setting C. 7.0 kV, 28 pulses, 1.6-second burst time, three cycles, 120-microsecond pulse width

Application of the electric field took <1 minute. The oocytes were immediately washed three times in phosphate-buffered saline solution and returned to the 5% carbon dioxide incubator in Whitten's media. After incubation the oocytes were examined.

Assessment of enzyme transfer. Oocytes and embryos were subjected to electric field-mediated transfer on setting C in Whitten's media containing 1 mg/ml of the enzyme horseradish peroxidase (Sigma). After washing three times in phosphate-buffered saline solution, the cells were fixed in 2.5% glutaraldehyde in phosphate-buffered saline solution. The enzyme substrate 3,3'-diaminobenzidine (Sigma) and 0.01% hydrogen peroxide was then added. The cells were mounted on slides and examined with phase contrast and confocal microscopy (Biorad, Cambridge, Mass.) for the presence of intracellular brown precipitate characteristic of the horseradish peroxidase-3,3'-diaminobenzidine-hydrogen peroxide reaction.1 These results were then compared with those obtained with lucifer yellow transfer on setting C. This was done to determine if the two methods of assessing transfer yielded similar results.

Controls to rule out possible endogenous enzyme substrate and endogenous peroxidase activity were run simultaneously with the experimental group. These controls were performed by excluding either the enzyme substrate (3,3'-diaminobenzidine-hydrogen peroxide) or the enzyme (horseradish peroxidase), respectively. Also, to rule out endocytosis, the cells were incubated in 1 mg/ml of horseradish peroxidase for 20 minutes without going through electric field-mediated transfer. These cells were then fixed and the substrate 3,3'-diaminobenzidine-hydrogen peroxide was added.

Assessment of Lucifer yellow transfer. A 1 mg/ml concentration of the fluorescent dye lucifer yellow (Sigma) in Whitten's media was used for electric fieldmediated transfer on each of the three sections (A, B,

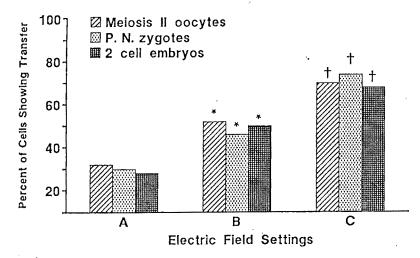


Fig. 1. Percentage of meiosis II oocytes, pronuclear zygotes, or two-cell embryos that showed evidence for molecular transfer of lucifer yellow dye after electric field-mediated transfer on setting A, B, or C. Asterisk, p < 0.01 when A compared with B; dagger, p < 0.01 when B compared with C. P.N., Pronuclear.

and C). After this procedure the cells were washed six times in phosphate-buffered saline solution, mounted on slides, and assessed for dye transfer with the use of fluorescence microscopy as described by Mir et al.2 Control oocytes were incubated in 1 mg/ml of lucifer yellow for 2 minutes, washed six times in phosphate-buffered saline solution, and compared with the oocytes that had undergone electric field-mediated transfer.

Assessment of germinal vesicle breakdown after electric field-mediated transfer. Germinal vesicle stage oocytes underwent electric field-mediated transfer on setting C. They were then incubated for 6 hours in a 5% carbon dioxide environment at 37° C. Light microscopic determination of germinal vesicle breakdown was then compared with control oocytes that had not been subjected to electric field-mediated transfer.

Assessment of viability after electric field-mediated transfer. Oocytes and embryos underwent electric field-mediated transfer on setting A, B, or C and were then incubated for 24 hours. After incubation they were placed in 10 µg/ml of the vital dye rhodamine 123 for 3 minutes and washed three times in phosphate-buffered saline solution. Fluorescence microscopy was used to assess incorporation of rhodamine 123 into mitochondria.3 These were compared with controls that had not undergone electric field-mediated transfer.

Assessment of fertilization and blastocyst transformation after electric field-mediated transfer. To assess the effects of electric field-mediated transfer on fertilization, meiosis II stage oocytes underwent the procedure at setting A, B, or C followed within 10 minutes by in vitro insemination with epididymal sperm. Fertilization rates for settings A, B, and C were compared with those of controls that had not under-

gone electric field-mediated transfer or had undergone electric field-mediated transfer without insemination (i.e., parthenogenetically activated oocytes).

Blastocyst transformation from in vivo and in vitro fertilized pronuclear stage zygotes and two-cell embryos after electric field-mediated transfer were assessed for all three settings and compared with simultaneously run controls that had not undergone electric field-mediated transfer.

Statistics. χ^2 Analysis of all groups was performed. The Bonferroni adjustment was used because multiple comparisons were analyzed for each group of data.4

Results

Molecular transfer rates. Lucifer yellow and horseradish peroxidase were used to determine the molecular transfer rate after electric field-mediated transfer on setting C. Transfer of the enzyme horseradish peroxidase with subsequent addition of 3,3'-diaminobenzidine-hydrogen peroxide yielded a dark brown precipitate within 64 of 90 (71%), 47 of 64 (73%), and 71 of 106 (67%) of meiosis II stage oocytes, pronuclear stage zygotes, and two-cell embryos, respectively. The difference between those groups was not significant. Intracellular fluorescence after lucifer yellow transfer was seen in 60 of 85 (70%), 60 of 81 (74%), and 54 of 80 (68%) for meiosis II stage oocytes, pronuclear stage zygotes, and two-cell embryos, respectively (not significant). Since horseradish peroxidase and lucifer yellow transfer rates were similar and lucifer yellow provided a direct means for evaluating transfer, it was used for the majority of the molecular transfer experiments. The results are shown in Fig. 1. With setting B the number of cells that showed dye transfer was 50 of 97 (52%), 44 of 95 (49%), and 45 of 94 (48%) for meiosis

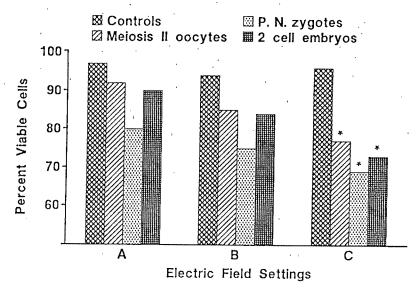


Fig. 2. Percentage of controls, meiosis II oocytes, pronuclear zygotes, or two-cell embryos that were viable after electric field-mediated transfer on setting A, B, or C. Asterisk, p < 0.01 when setting A, B, or C is compared with controls. P.N., Pronuclear.

II stage oocytes, pronuclear stage zygotes, and two-cell embryos, respectively (not significant). With setting A the number of cells that showed dye transfer was 24 of 75 (32%), 21 of 70 (30%), and 22 of 78 (28%) for meiosis II stage oocytes, pronuclear zygotes and two-cell embryos, respectively (not significant).

It was noted in all our transfer experiments that when dye of enzyme transfer occurred in a two-cell embryo, both cells contained the transferred macromolecules. We also noted that control oocytes and embryos that had not undergone electric field-mediated transfer but were incubated in horseradish peroxidase or lucifer yellow did not show evidence of molecular transfer.

There was a statistically significant increase in molecular transfer when setting A was compared with B and setting B was compared with C (p < 0.02).

Viability after electric field-mediated transfer. The results of our viability testing 24 hours after electric field-mediated transfer are shown in Fig. 2. With setting A the number of viable cells was 60 of 62 (97%), 70 of 76 (92%), 85 of 106 (80%), and 65 of 72 (90%) for controls, metaphase II stage oocytes, pronuclear stage zygotes, and two-cell embryos, respectively.

With setting B the number of viable cells following electric field-mediated transfer was 48 of 51 (94%), 61 of 72 (85%), 52 of 69 (75%), and 49 of 58 (84%) for controls, meiosis II stage oocytes, pronuclear stage zygotes, and two-cell embryos, respectively.

With setting C the number of viable cells after electricfield-mediated transfer was 47 of 49 (96%), 46 of 60: (77%), 42 of 61 (69%), and 43 of 59 (73%) for controls, meiosis II stage oocytes, pronuclear stage zygotes, and two-cell embryos, respectively.

Only setting C had a significantly lower viability rate than the controls that had not undergone electric fieldmediated transfer (p < 0.01).

Germinal vesicle breakdown. The rate of germinal vesicle breakdown after electric field-mediated transfer on setting C was compared with that of controls. Germinal vesicle breakdown occurred in 80 of 102 (78%) of oocytes exposed to electric field-mediated transfer versus 79 of 95 (83%) of controls (not significant).

Fertilization rates after electric field-mediated transfer. It was noted that 85 of 157 (54%) of meiosis II stage oocytes exposed to the electric field without subsequent insemination developed into normalappearing two-cell embryos. The majority of these parthenogenetically activated oocytes were arrested at the two-cell stage. When the oocytes were inseminated after electric field-mediated transfer, 85 of 131 (65%) developed to the two-cell stage, but only 11% developed into blastocysts.

Blastocyst transformation after electric fieldmediated transfer. The transformation from two-cell embryos to blastocyst was evaluated after electric fieldmediated transfer in pronuclear stage zygotes and twocell embryos (Fig. 3). The blastocyst transformation rates for pronuclear stage zygotes on settings A, B, and C were 46 of 94 (49%), 36 of 78 (46%), and 31 of 69 (45%), respectively (not significant). Sixty-four of 88 (73%) control zygotes not exposed to electric fieldmediated transfer went from two-cell embryos to blastocyst, and this was statistically higher than all the experimental groups (p < 0.01).

Blastocyst transformation after electric field-me-

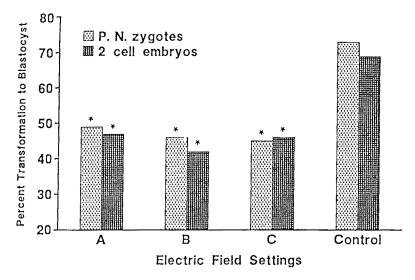


Fig. 3. Percentage of pronuclear stage zygotes or two-cell embryos that progressed normally from two-cell stage to blastocyst after electric field—mediated transfer on setting A, B, or C. Asterisk, p < 0.01 when pronuclear zygotes or embryos are compared with controls. P.N., Pronuclear.

diated transfer of two-cell embryos resulted in 79 of 168 (47%), 33 of 78 (42%), and 37 of 81 (46%) for settings A, B, and C, respectively (not significant). Fifty-two of 77 (68%) control two-cell embryos not exposed to electric field—mediated transfer transformed to blastocyst. The controls showed a significantly higher blastocyst transformation than all the electric field—mediated transfer groups (p < 0.01).

Comment

Electric field—mediated transfer evolved from the observation that cells could be induced to fuse when exposed to an electric field.⁵ This idea was modified to allow molecular transfer through permeation structures created by the electric field.⁶ The mechanism by which this works is unknown, but in theory, when an electric current is superimposed on the resting membrane potential, a critical threshold value is reached, at which point there is a dramatic and reversible increase in conductivity and permeability of the cell membrane. When the electric current is stopped, the permeation structures rapidly seal because of the fluid nature of the membrane, and the resting membrane potential is restored.

Until now almost all electric field—mediated transfer has involved deoxyribonucleic acid transfection and transformation in bacteria, plants, and other mammalian cell lines. Recently, we have expanded its use to the transfer of functional enzymes into human oocytes with an efficiency of 87% and a viability of 89%.

In this study we were able to show that transfer of the enzyme horseradish peroxidase into mouse oocytes can be accomplished in up to 73% of oocytes and that the functional activity of this enzyme is retained. The percentage of oocytes and embryos that showed molecular transfer varied depending on the electric field setting A, B, or C (Fig. 1). Higher voltage settings yielded higher transfer rates. This is in agreement with other studies using different cell lines.^{2, 10} The molecular transfer rate was similar for metaphase II oocytes, pronuclear stage zygotes, and two-cell embryos when the same setting was used.

Setting C was the original setting found to be suitable for transfer into mouse and human oocytes. Most of the adverse effects on viability are from increased voltage. Therefore the voltage was decreased for our new settings, A and B. We knew that lower voltage would have an adverse effect on the transfer rate. To compensate for this we increased the number of cycles to which the cells were exposed. It was hoped that this would maintain the same transfer rate as setting C and increase the viability. However, this was not the case. The lower voltage settings (A and B) had a lower transfer rate but did maintain a higher viability.

Meiotic maturation as assessed by germinal vesicle breakdown did not seem to be affected by electric field—mediated transfer; 84% of the controls and 79% of the oocytes showed germinal vesicle breakdown within 6 hours (not significant). Only setting C was used for this portion of the experiment because it was the most destructive in our viability testing and therefore most likely to have a detrimental effect.

Our test for viability used the vital dye rhodamine 123, which incorporates into mitochondria of living cells.³ As the voltage setting was increased, the viability decreased as shown in Fig. 2. However, only setting C showed a statistically significant decrease in viability when compared with that of controls. This decrease in

viability seen with high voltage has been reported by other investigators using different cell lines.2, 9, 10

Fertilization after electric field-mediated transfer was performed in vitro with meiosis II stage oocytes. We noted a 55% parthenogenetic activation rate when meiosis II stage oocytes were exposed to the electric field without subsequent insemination. When these meiosis II stage oocytes were inseminated after electric field-mediated transfer, 65% were at the two-cell stage 24 hours later. However, only 11% developed to blastocyst. These results suggest that the majority of oocytes that reach the two-cell stage probably were parthenogenetically activated and that some block to fertilization was present. Onodera and Tsunoda¹¹ recently reported similar parthenogenetic activation rates in mouse and rabbit oocvtes. Alterations in the cell membrane potential or an increase in intracellular calcium can cause parthenogenetic activation and subsequent release of the cortical granules. 12 This could explain the decreased fertilization potential of these oocytes after electric field-mediated transfer.

The transformation rate from two-cell embryos to blastocyst was evaluated in the pronuclear zygotes and the two-cell embryos after electric field-mediated transfer. Blastocyst transformation rates were similar for all three settings and for both cell types (i.e., two cell embryos and pronuclear zygotes). However, pronuclear stage zygotes and two-cell embryos both showed a statistically significant decrease in blastocyst transformation after being exposed to electric fieldmediated transfer when compared with controls (p < 0.01) (Fig. 3).

The enzyme horseradish peroxidase (40 kd) and the dye lucifer yellow (457 d) were used to evaluate molecular transfer. Horseradish peroxidase was used because it is not normally found in oocytes, and we wanted to determine if electric field-mediated transfer had any detrimental effect on the functional activity of an enzyme. The brown precipitate formed within the cells by the horseradish peroxidase-3,3'-diaminobenzidine-hydrogen peroxide reaction shows that the enzyme remained functional after electric field-mediated transfer.

Lucifer yellow transfer is an established technique in other cell lines and was used to confirm the results obtained with the horseradish peroxidase-3,3'-diaminobenzidine-hydrogen peroxide method that we developed.1,2 Similar results were obtained with both techniques (74% vs 73% molecular transfer for lucifer yellow and horseradish peroxidase, respectively, on setting C with pronuclear stage zygotes). Horseradish peroxidase and lucifer yellow are minimally permeable into mouse oocytes and embryos if left in solution for a long enough duration. In all of our electric field-mediated transfer experiments the cells were exposed to lucifer yellow or horseradish peroxidase for no more than 1 to 2 minutes. In this short amount of time no passive transport of these molecules is noted in control oocytes or embryos. This suggests than any lucifer yellow or horseradish peroxidase present within the cell has been actively transferred into that cell because of the electric field. There is nothing unique to the lucifer yellow or horseradish peroxidase molecules that enables them to be transferred more easily than any other molecules. Therefore introduction of other more significant molecules such as immunoglobulins to specific cell proteins or genetic material also can be performed with this technique. Currently we do not know the maximum molecular size that can be transferred into oocytes. However, a 150 kb linear, double-standard deoxyribonucleic acid has been electrotransfected into CV-1 cells.13 There are many biologic variables that affect electric field-mediated transfer such as field strength, pulse wave shape and duration, ionic concentration, pH, temperature, cell type, molecular configuration, and charge.10 A change of any of these variables can affect transfer rates and viability. It is therefore essential to carefully control for all these parameters.

Confocal microscopy was initially used to confirm the intracellular location of the precipitate formed by the horseradish peroxidase-3,3'-diaminobenzidinehydrogen peroxide reaction. This microscope, by the nature of its optics, enables the viewer to see thin sections (0.67 µm) of the specimen, similar to a computerized axial tomogram, thus confirming an intracellular location.

Since electric field-mediated transfer allows direct access to the cytosol, it potentially could be used to study intracellular physiologic characteristics, regulatory mechanisms, and cell replication in early embryos. Directing or controlling various oocyte or embryo functions is another possibility. Its role in the field of human reproduction is yet to be established, but it has been used to induce the acrosome reaction in human sperm and to transfer enzymes into human oocytes. 1, 14 In the future it could potentially be used to transfer genetic material into embryos diagnosed by embryo biopsy as having certain autosomal recessive or sex-linked disorders or for production of transgenic animals. It will be important to know if normal live offspring can be produced from embryos or oocytes exposed to electric field-mediated transfer. We assume that this is possible since we have shown that normal blastocyst development can occur after this procedure. Investigations are currently under way to confirm this.

In conclusion, electric field-mediated transfer can be used to transfer nonpermeable molecules into mouse oocytes, zygotes, and embryos. The molecules transferred into the oocytes remained functional. Increasing the electric field strength yielded a higher molecular transfer rate but a lower viability rate. Molecular transfer and viability were not dependent on the stage of the oocyte (i.e., metaphase II oocyte, pronuclear stage zygotes, or two-cell embryos). Meiotic maturation does not appear to be affected by electric field—mediated transfer. Blastocyst transformation after electric field—mediated transfer was not dependent on electric field strength but was statistically lower than that of controls. Electric field—mediated transfer of meiosis II metaphase oocytes causes a high rate of parthenogenetic activation and therefore significantly decreases in vitro fertilization rates. Electric field—mediated transfer is a simple, relatively atraumatic tool that can be used to study intracellular functions of oocytes and the developmental process of early embryos.

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Forward shift in the initiation of the nocturnal estradiol surge in the pregnant baboon: Is this the genesis of labor?

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Daily (9 AM and 6 PM) blood samples were obtained from the inferior vena cava during the last trimester of pregnancy in a tethered baboon model. In addition, three 24-hour (hourly blood sampling) studies were performed at days 143 to 147, 158 to 162, and 172 to 177 of pregnancy. Dramatic 24-hour rhythms in progesterone and estradiol were detected, with both steroids surging nocturnally. Early in the third trimester the estradiol surge followed the progesterone surge. However, approximately 10 to 12 days before delivery, the initiation of the nocturnal estradiol surge shifted forward, thus preceding the progesterone surge. This forward shift in the estradiol surge created a daily (3 to 5 hours) window of elevated estradiol-to-progesterone ratio and appears to coincide with the initiation of nocturnal uterine contractions. The nocturnal uterine contractions can be inhibited by an oxytocin antagonist. We hypothesize that this forward shift in the initiation of the estradiol surge induces nocturnal uterine contractions by oxytocin release and/or increase in uterine oxytocin receptors and generates molecular messages that are the genesis for labor and delivery in the baboon. (AM J OBSTET GYNECOL 1991;165:1487-98.)

Key words: Estradiol, progesterone, uterine contractions, pregnancy, baboon

In nonprimate animals such as sheep, cows, goats, and rats parturition is preceded by a precipitate decrease in progesterone and a rise in estradiol in the blood. The increase in the estradiol/progesterone ratio can explain many of the changes that occur at delivery, such as an increase in uterine oxytocin receptor number, uterine prostaglandin production, and release of maternal oxytocin. In contrast, in primates such as humans, chimpanzees, hesus monkeys, and baboons, the ratio of estradiol to progesterone remains relatively constant through delivery.

The baboon is an excellent model for studying the endocrinology of human pregnancy because of its physiologic and anatomic similarity. The baboon has a hemochorial and monodiscoidal placenta and a fetal-placental unit in which the fetal adrenals contribute the major precursor for estrogen synthesis by the placenta. The one major dissimilarity in baboons from humans and great apes (gorilla, orangutan, and chimpanzee) is their inability to produce estriol. The major plasma estrogen is estradiol.⁶

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In this study we report the daily changes in plasma estradiol and progesterone throughout the last trimester of pregnancy and show that they have a significant 24-hour rhythm. Early in the third trimester estradiol and progesterone surge nocturnally, with the rise in estradiol following that of progesterone. However, as pregnancy progresses, the initiation of the estradiol surge shifts forward and precedes the progesterone surge by 3 to 5 hours. This shift in estradiol appears to coincide with the initiation of nocturnal uterine activity. Thus, although the ratio of plasma estradiol to progesterone over a 24-hour period does not change dramatically with increasing gestational age, a 3- to 5hour period develops where the ratio of estradiol to progesterone is elevated. We propose that this daily window of elevated estradiol/progesterone ratio initiates nocturnal uterine contractions by induction of biochemical alterations and generates other molecular messages that lead to labor and delivery.

Material and methods

Timed pregnant baboons (*Papio anubis*) from the breeding colony of the Biological Resource Laboratory at the University of Illinois were used in these studies. The animals were kept under controlled lighting conditions (12 hours light/12 hours dark) with lights on at 6 AM and fed food (Purina Primate Chow, fruit, nuts) once a day and water as desired. Normal delivery occurs at 184 ± 2 days. The protocol for this report has been approved by the Animal Care Committee at the University of Illinois.

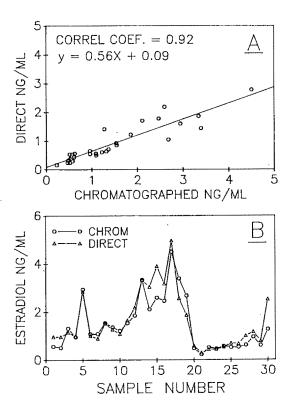


Fig. 1. Relationship between direct assay of estradiol in plasma samples by radioimmunoassay compared with prior extraction and chromatography of plasma. A, Correlation and leastsquares regression line between two assay methods for 30 plasma samples. B, Results of correcting direct assay data to 100% and comparing these answers with extracted-chromatographed samples. After correction results did not differ between two methods (p > 0.05).

The surgical procedure has been previously described in detail.7 Briefly, all baboons were operated on between 125 and 135 days of pregnancy. A midline abdominal incision was made to expose the uterus. Two 0.86×1.32 mm vinyl cannulas approximately 15 inches in length were inserted into the amniotic fluid by a 16-gauge needle acting as a trocar. The cannulas were exteriorized by a midlateral flank incision. A second incision was made in the groin area of the leg just above the external femoral vein and artery. Vinyl cannulas similar to those in the amniotic fluid were placed into each vessel so that the tip resided in the vena cava or aorta just above the bifurcation (approximately 8.5 inches in length). These cannulas, plus those from the amniotic fluid, were led subcutaneously to a middorsal area just inferior to the scapula. All skin incisions were closed with 2-0 Vicryl suture. A nylon mesh jacket (Alice King Chatham Medical Art, Los Angeles) was placed on the baboon, and the cannulas were led out through a flexible stainless steel tether attached to the back of the jacket. The tether, 24 inches in length, was attached to a punched-out training swivel on the side of the cage.

This technique allows the cannulas to be directly attached to pressure transducers and infusion pumps.8 The amniotic fluid cannulas were attached to P23id Gould pressure transducers, and pressure changes were recorded on a model 7 Grass polygraph (both from Grass Instruments, Quincy, Mass.). In addition, the analog signal was digitized with a Data Translation 2811H board on a Dell PC Limited 286 computer. The analog signal was sampled every second. The data were expressed as contractile force (Frequency × Mean amplitude) per 30-minute interval. The cannulas in the femoral vein and artery were attached to a syringe on a Harvard infusion pump. Heparin (100 U/ml) and cefazolin (71.4 mg/ml; Ancef, Smith Kline & French, Philadelphia) in saline solution were infused at a rate of 0.582 ml/hr to keep the cannulas patent and to deliver 1 gm/day cefazolin to the animals. After the first week the cefazolin was reduced to 0.5 gm/day.

Experimental protocol. Each day through delivery at 9 AM and 6 PM 10 ml of blood was withdrawn from the femoral vein cannula in five baboons. Blood samples were collected over ice and centrifuged at 4° C for 20 minutes at 2000 revolutions/min, the plasma was stored at -70° C until analysis. In addition to the daily samples, three 24-hour studies were performed at days 143 to 147, 158 to 162, and 173 to 177 in four of the five baboons. However, only one baboon was studied at day 175 since the other three delivered before this stage of pregnancy. Five milliliters of blood was collected every hour as described above for the daily samples.

Steroid radioimmunoassays. The radioimmunoassays for estradiol and progesterone have been previously described. 9, 10 Plasma progesterone was extracted with petroleum ether, evaporated, and assayed. Tritium-labeled progesterone was added at the beginning of the extraction procedure for estimating procedural losses, and all values have been corrected for such losses. The average recovery for the assay was 73% with intraassay and interassay variations of 5% and 6%, respectively.

Estradiol was measured directly in the plasma after the samples were diluted 1:5 in assay buffer. In preliminary studies the estradiol results were compared with those of a previously validated assay that consisted of ether-extracted, column-chromatographed plasma samples.10 The estradiol values from the direct assay of 30 diluted samples were 56% of those for the same extracted-chromatographed samples. However, the correlation coefficient of the estimated values between these two assays was 0.92 (Fig. 1, A) and was highly significant (p < 0.05). When the plasma estradiol levels were corrected for the 44% under estimation, the results closely compared with the extracted-chromatographed values (Fig. 1, B). No differences (p > 0.05)

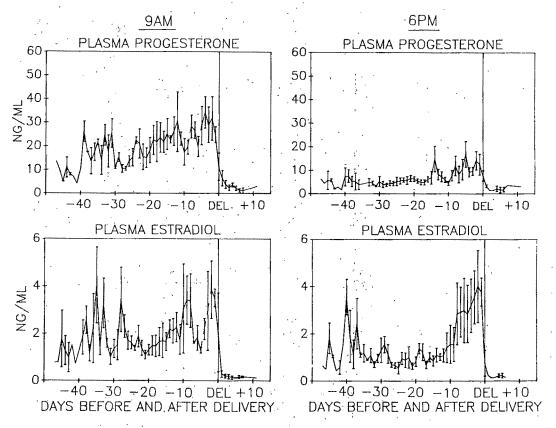


Fig. 2. Daily 9 AM and 6 PM progesterone and estradiol plasma levels adjusted to delivery during last trimester in pregnant baboons (n = 5; mean \pm SEM).

were detected when corrected values were compared by paired t test with the extracted-chromatographed values. Thus, although the values reported here are about 56% of the actual values, the patterns of variation are highly correlated with the actual values. The intraassay and interassay variations for this assay were 6% and 10%, respectively.

Statistical analysis. Data were analyzed by repeat analysis of variance, and, when significant, differences between means were detected by Tukey's test. In addition, the daily blood samples were analyzed by correlation and regression analysis. Finally, where appropriate, paired t tests were performed. ¹¹

Results

Changes in the daily 9 AM and 6 PM plasma estradiol and progesterone levels adjusted to the day of delivery in five baboons are illustrated in Fig. 2. There was substantial variation in the steroid levels during the first 10 to 15 days. This variation was due to the range in days during which the animals were operated on and to the effects of surgery on plasma steroids. Our study, plus that in the rhesus monkey, 12 show that progresterone and estradiol are elevated for 3 to 5 days after surgery. Thus, for the purpose of regression analysis only, the last 25 days before delivery was examined.

The correlation coefficient, regression equation, and level of statistical significance for the 9 AM and 6 PM progesterone levels versus the day of pregnancy before delivery were r = 0.29, y = 0.51x + 17.28, p < 0.007and r = 0.41, y = 0.38x + 3.73, p < 0.0001, respectively. Similarly, for the 9 AM and 6 PM plasma estradiol levels the results were r = 0.37, y = 0.074x + 1.138, p < 0.0003 and r = 0.54, y = 0.13x + 0.057, p < 0.00030.0001, respectively. The 9 AM plasma progesterone value was greater than the paired 6 PM value 91% of the time, and this was highly significant (p < 0.001) by paired t test. The estradiol levels showed more variation. The 9 AM values were greater than the paired 6 РМ value only 68% of time, and this was not significant (p > 0.05). Both progesterone (p < 0.001) and estradiol (p < 0.001) showed a significant effect of day of pregnancy in the repeat analysis of variance, and this is reflected in the statistically significant positive regression slopes.

Examples of plasma progesterone and estradiol levels in 24-hour studies in individual baboons are shown in Figs. 3 and 4. Fig. 3 shows the results of three 24-hour studies in a single baboon and compares changes in progesterone and estradiol to uterine contractile force (Frequency × Mean amplitude per 30 minutes) at 145, 160, and 175 days of pregnancy. There is a substantial

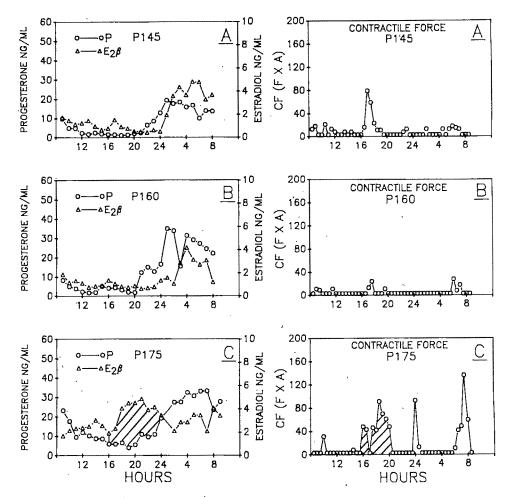


Fig. 3. Hourly fluctuations in steroids and contractile force over 24 hours at three stages of pregnancy in baboon 4953. At days 145 and 160 (A and B) progesterone surged nocturnally, followed by estradiol. However, at day 175 (C) initiation of estradiol surge shifted forward, preceding progesterone surge by 4 hours. Hour at which estradiol surge began coincides with initiation of nocturnal uterine activity (hours refer to 24-hour clock).

24-hour rhythm at each of the days studied, with progesterone and estradiol surging in the evening. At days 145 and 160 the estradiol surge follows the progesterone surge by several hours. However, by day 175 the initiation of the estradiol surge shifts forward so that it now precedes the progesterone surge by 3 to 4 hours. Coincident with this forward shift in the estradiol surge is the initiation of nocturnal uterine activity (Fig. 3, C).

Similar results are shown in another baboon in Fig. 4. At day 146 the nocturnal plasma estradiol surge follows the progesterone surge. However, at day 160 the initiation of the estradiol surge shifts forward and thus precedes the progesterone surge by 4 hours. This surge in estradiol coincides precisely with the increase in uterine contractile force activity. In this particular animal the uterine contractions were of such magnitude that an oxytocin antagonist produced in our laboratory, $^{7.13}$ [β -mercapto- β , β -cyclopentamethylene propionic acid,

D-Trp2, Phe3, Ile4, Arg8]oxytocin, was given as a single intravenous bolus injection of 3000 nmol to prevent delivery during the experiment. The oxytocin antagonist caused an immediate cessation in uterine contractions, but the estradiol surge appeared to be unaltered. No additional oxytocin antagonist was given, and this animal delivered spontaneously 7 hours after the completion of this study. The newborn was alive and appeared healthy and normal. The 24-hour experiment at day 175 could not be performed in this animal because of the delivery. The difference between the timing of the estradiol surges at day 160 of pregnancy in Figs. 3 and 4 represents the relationship of the day of pregnancy to delivery. The animal in Fig. 3 delivered at day 182; therefore day 160 was 22 days from delivery, which is before the shift in the estradiol surge would be expected to occur. In contrast, the baboon in Fig. 4 delivered hours after the end of the study on day 161.

In Figs. 3 and 4 the uterine activity at 12 midnight

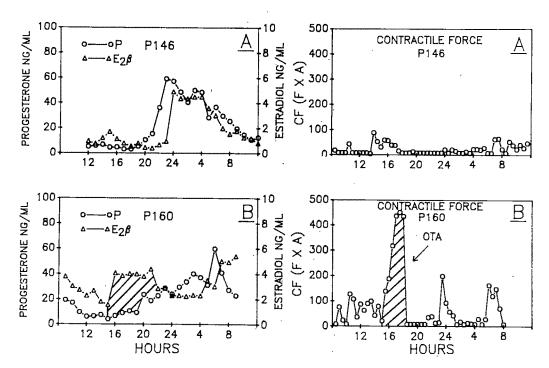


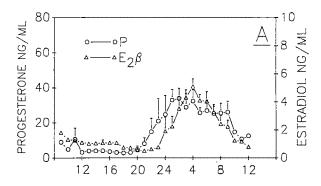
Fig. 4. Hourly fluctuations in steroids and contractile force over 24 hours at days 146 and 160 of pregnancy in baboon 4689. Similar to baboon 4953 in Fig. 3, forward shift in initiation of estradiol surge coincided with dramatic increase in uterine contractile force. Uterine contractile force was of such intensity that oxytocin antagonist (OTA), produced in our laboratory, was administered as a single bolus injection of 3000 nmol to prevent delivery during 24-hour study. Oxytocin antagonist caused immediate inhibition of uterine contractions, but estradiol surge was unaltered. This baboon delivered 7 hours after end of study (hours refer to 24-hour clock).

and 6 AM does not appear to correlate with the estradiol surge. However, the 12 midnight values are transient, particularly the one in Fig. 3 that consists basically of one point. The 12 midnight peak in Fig. 4 is slightly less transient, but it must be recognized that this animal was most likely in labor and that only the administration of the oxytocin antagonist prevented delivery. The uterine activity at 6 AM coincides with the turning on of the lights in the room (lights off at 6 PM and on at 6 AM). It is not unusual to see transient uterine activity related to this phenomenon or when the animals are fed, particularly if they are close to delivery. In addition, the plasma estradiol levels in the baboon in Fig. 4 remained elevated and appeared to be surging 6 AM; this might have contributed to the uterine activity at this time.

Fig. 5 summarizes the 24-hour studies for four baboons by comparing studies >10 days before delivery (Fig. 5, A) with those <10 days before delivery (Fig. 5, B). Both progesterone and estradiol showed significant diurnal variation (p < 0.001). The estradiol surge was statistically significant but the comparison of the 9 AM and 6 PM estradiol values (see above) was not, because the latter values represent time periods closer to the midday nadir in estradiol. It is the peak in the nocturnal estradiol surge that differs significantly from

the nadir in the afternoon samples. The data for these 24-hour steroid fluctuations were fitted to a tenth-order polynomial, and this resulted in correlation coefficients of >0.99 for all polynomials. First-order derivatives of these polynomials determined the rate of change of the plasma steroid levels and provided an estimate of the initiation and peak of these nocturnal surges. These data indicated that >10 days before delivery the estradiol surge follows the progesterone surge by about 3 hours. The progesterone surge begins about 7 PM and peaks at 2:30 AM the following morning. The estradiol surge begins at 10 PM and peaks at 4:30 AM the next day. In contrast, <10 days before delivery the initiation of the estradiol surge shifts forward 6 hours and precedes the progesterone surge by 2 to 3 hours. The estradiol surge being about 4 PM and peaks at 1:30 AM the next day. The progesterone surge begins at 6 PM and attains a peak at 5 AM the following day.

The 24-hour studies show a substantial amount of variation because of the different times when the estradiol and progesterone surges begin, the different levels of estradiol and progesterone in the plasma, and the small number of observations. Averaging together such data gives a misleading picture as to the characteristic shape and relationship of the estradiol surge to the progesterone surge. The estradiol surge has more



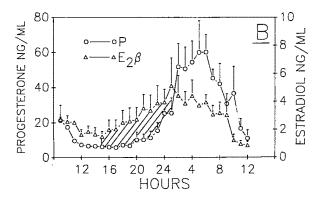


Fig. 5. Hourly fluctuations in steroids (mean \pm SEM) over 24 hours at >10 and <10 days before delivery. A, >10 days before delivery nocturnal estradiol surge followed progesterone surge. B, <10 days before delivery initiation of estradiol surge shifted forward and preceded nocturnal progesterone surge (N = 4; hours refer to 24-hour clock).

of a square-wave appearance (e.g., Figs. 3 and 4) when an individual baboon is examined. In an attempt to adjust for this variation the data were reanalyzed by comparing all values relative to initiation of the estradiol surge and examining the data ±6 hours of this surge. These results are shown in Fig. 6. The initiation of the estradiol surge (Figs. 5 to 7) was determined by calculating the first derivative for each time period by the use of n, n-1 and n+1 for the quadratic equation. The time period immediately preceding the first peak derivative past the midday nadir for plasma estradiol was considered time 0. If the derivative immediately preceding the peak derivative differed by no more than 0.03 (thus indicating a significant change), then the time period immediately preceding this hour was considered time 0. When expressed in this form, the mean estradiol surge appears more as a square wave. At >10 days before delivery the estradiol surge began 2 hours after the progesterone surge (Fig. 6, A), but at <10 days before delivery the initiation of the estradiol surge preceded the progesterone surge by 4 hours (Fig. 6, B). Thus the forward shift in the initiation of the estradiol surge compared with the progesterone surge was approximately 6 hours. Statistical analysis of these adjusted data indicated that both the progesterone and estradiol surges were significant (p < 0.01). Three of the four animals in the study showed a shift in the initiation of the estradiol surge of 4 to 5 hours compared with the start of the progesterone surge. In the fourth animal the initiation of the estradiol surge was advanced only about 1 hour. The fourth animal was 7 days from delivery, which was the maximum in time difference from delivery in the four animals in the study.

Similar to the steroid data, the contractile force was adjusted relative to the initiation of the estradiol surge; these results are shown in Fig. 7. The variation is very large because of the differences in contractile force between animals and the small number of animals studied. However, it can be seen that the rise in the contractile force coincides with the rise in estradiol. Of the four animals represented by these data, one was in labor, one showed no uterine contractile activity, and the other two had moderate activity.

Comment

Several important observations have been made in this study. First, the daily 9 AM and 6 PM progesterone and estradiol levels showed significant (p < 0.05) increases during the last 25 days before delivery, with the 9 AM progesterone and 6 PM estradiol levels showing the most dramatic increases. In addition, the 9 AM plasma progesterone level was higher (p < 0.05) than the 6 PM value 91% of the time. In contrast, no statistical difference in plasma estradiol levels was detected between 9 AM and 6 PM with advancing gestational age. The significant increase in plasma progesterone levels during the last trimester is in contrast to previous reports in the baboon,14,15 which suggested that progesterone reached a plateau by day 60 of pregnancy and remained constant until delivery. We believe this discrepancy in results can be explained by the time of day the blood samples were collected and is related to the 24-hour rhythm of plasma steroid concentrations (Fig. 5). In these previous reports a single daily blood sample was collected between 9 and 11 AM14 and 11 AM and 1 PM.15 Under the lighting regimen in our baboon colony these times would be close to the nadir in the progesterone surge (11 AM to 8 PM). The increase in the daily 9 AM plasma progesterone levels is more similar to that in humans2 than has been previously suggested from single daily blood samples obtained in the other baboon studies. 6, 14, 15 Considering the dramatic 24-hour rhythms in plasma progesterone and estradiol, variations in steroid levels as a result of single daily blood sampling in the baboon must be viewed with caution, since they may simply reflect shifts in the steroid surges

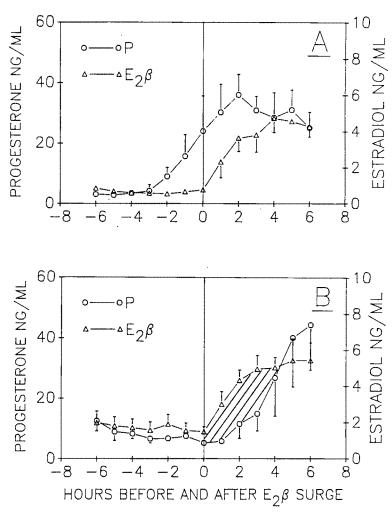


Fig. 6. Progesterone and estradiol in Fig. 5 were adjusted to initiation of estradiol surge and analyzed for 6 hours before or after this surge. When presented in this form, estradiol surge appears more as a square wave and reflects more accurately the characteristic appearance in individual baboons. Total shift in estradiol surge from >10 days (A) to <10 days (B) before delivery was about 6 hours (mean \pm SEM; N=4; hours refer to 24-hour clock).

and should not be perfunctorily interpreted as alterations in synthesis.

Similar to previous reports^{5, 14} in the baboon, significant increases in the daily plasma estradiol levels were seen during the last trimester. Most intriguing in this study was the rapid increase in the 6 PM plasma estradiol levels during the last 12 days before delivery. We believe this increase is related to the forward shift in the initiation of the estradiol surge into the time frame (6 PM) when the daily blood samples were collected. This increase in daily plasma estrogen during the last trimester is similar to reports for humans2 and rhesus monkeys.4

The second significant observation in this study is the identification of a 24-hour rhythm in plasma progesterone and estradiol during the last trimester of pregnancy. This has not been reported previously in the baboon except in abstract form. 16, 17 Plasma estradiol

and progesterone showed a significant 24-hour rhythm with nocturnal surges beginning between 7 and 10 PM and reaching peaks between 2:30 and 4:30 AM the next morning. Similar 24-hour rhythms have been reported in rhesus monkeys for progesterone and estradiol. 18, 19 However, in one study¹⁸ the estradiol rhythm was not statistically significant. In humans, 24-hour rhythms for estriol and progesterone were detected at 30 to 31 and 34 to 35 weeks of pregnancy but not at 38 to 39 weeks.20.21 In both human and rhesus monkey studies the incrementation from baseline to peak plasma steroid levels was not as great, and thus the differences not as evident, as in this study. There are several potential explanations for these differences. First is the site of blood sampling. The blood samples in this study were obtained from a cannula inserted in the inferior vena cava at a point past where the uterine veins empty.

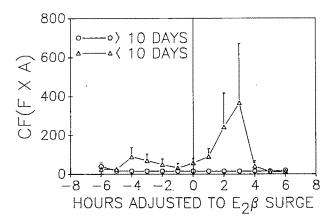


Fig. 7. Uterine contractile force adjusted to initiation of estradiol surge as represented in Fig. 6. Initiation of uterine contractile force <10 days before delivery coincided with initiation of estradiol surge (mean \pm SEM; N=4; hours refer to 24-hour clock).

Thus the progesterone and estradiol concentrations may reflect more the level of steroid hormones emanating from the uterus and to a lesser degree the results of peripheral metabolism, metabolic clearance, and dilution. In the human studies^{20,21} the blood samples were taken from the antecubital vein, whereas in the rhesus monkey reports¹⁸ the samples were from the aorta through a cannula placed in the femoral artery. We have performed 24-hour studies comparing aortavena cava differences in steroid levels (unpublished observations) in the pregnant baboon. Although the vena cava plasma steroid levels are often greater than the aortic concentrations, particularly for progesterone, substantial 24-hour rhythms are detected in both sources of blood. Thus this does not appear to be the only explanation for the more dramatic 24-hour steroid rhythms in this study, compared with those reported in the rhesus monkey and human. Second, species divergencies may contribute to the discrepancies in results. The progesterone and estradiol levels in rhesus monkey blood are much lower than in baboon and human blood. In addition, estriol, which is unique to humans and great apes, may have a role that is singularly important in these species. Finally, in the rhesus monkey reports18, 19 the studies were conducted earlier in pregnancy, when the maternal contribution of precursor to placental estrogen production is greater and may dilute the effect of fetal adrenal secretions on maternal estrogen levels, and the samples were collected less frequently (every 3 hours); thus the averaging of the values may abate the steroid rhythms by introducing more variation into the results.

In this study the timing in the initiation and peak of the steroid surges varies to some degree, compared with the rhesus monkey reports. The lighting regimen in the monkey studies was a cycle of 16 hours light/8 hours dark compared with this study of 12:12 hours. This may explain the dissimilarities in the timing of the peaks and valleys of the progesterone surge between these studies. Alterations in the light/dark cycle regimen have been reported to alter the timing of the nocturnal uterine activity and steroid hormone rhythms in the rhesus monkey.^{22, 23}

A rationale for the 24-hour rhythm in maternal plasma progesterone is less obvious than for estradiol. The precursor for placental progesterone is pregnenolone. It is possible that the fetal adrenals contribute to placental progesterone on activation of the fetal adrenals and increased pregnenolone secretion. Also, it is conceivable that the AM cortisol displaces progesterone from corticosteroid-binding globulin, contributing to enhanced clearance of free progesterone and consequently a reduction in plasma progesterone levels. There is evidence for this possibility. 19 A third explanation is that progesterone production may be dependent on estrogen. Administration of MER-25, an antiestrogen, to pregnant baboons reduced maternal progesterone by 50% and in vitro blocked the conversion of cholesterol to pregnenolone.24 Thus the rhythm in progesterone may be dependent on the estradiol rhythm. Finally, the metabolic clearance rate of progesterone appears to be influenced by cortisol, and this may contribute to the rhythm in progesterone levels.25 Obviously, further studies are required before a definitive explanation can be provided for the 24-hour rhythm in maternal progesterone. Clinically the recognition of the 24-hour rhythms in maternal plasma steroids might be important, for they suggest that assessment of fetal function and the fetal-placental unit dynamics may require obtaining at least two blood samples per day, one around midnight to 4 AM to establish the peak in the steroid surge and the other in the early afternoon (12 AM to 3 PM) for comparison with baseline values.

The third, and most important, observation in this study is the forward shift in the initiation of the nocturnal estradiol surge during the last 10 to 12 days of pregnancy, so that, unlike earlier in pregnancy, it precedes the nocturnal progesterone surge. This forward shift results in a period of 3 to 5 hours every day when the ratio of estradiol to progesterone is elevated, which seems to coincide with the beginning of nocturnal uterine activity. The observation that an oxytocin antagonist produced in our laboratory can inhibit this uterine activity (Fig. 2) without altering the pattern of the estradiol surge suggests that the surge is not dependent on enhanced uterine contractile activity but rather that enhanced uterine contractile activity might be dependent on the estradiol surge. In addition, these data indicate that oxytocin is a primary regulator of nocturnal uterine contractions, because of either an increase in oxytocin release, an enhanced uterine sensitivity to oxytocin, or both. We have previously reported that the oxytocin antagonist is a potent inhibitor of nocturnal and labor uterine contractions in the pregnant baboon.7 The data in this study suggest that estradiol is correlated with, and might be responsible for, the oxytocin effect. There are numerous reports in the literature indicating that estradiol is related to increased uterine activity12, 26 and can increase oxytocin release and uterine oxytocin receptor number. 27-29 Studies are in progress in our laboratory to determine the relationship among estradiol, oxytocin, and uterine contractions.

Although 24-hour studies in pregnant humans at different stages of pregnancy did not reveal a forward shift in the initiation of the nocturnal estradiol surge,20 close examination of this datum indicates a trend is present. For example, at weeks 34 to 35 the initiation and peak of the estriol surge occur about 4 hours earlier, compared with the estriol surge at weeks 30 to 31.20 A major difference between the human studies and this study is that the subjects examined at each stage of pregnancy in those experiments were not the same. This may have introduced more variation into those studies and obfuscated differences that might have been present.

The forward shift in the initiation of the estradiol surge also can explain the sharp increase in the 6 PM. plasma estradiol levels during the last 10 to 12 days before delivery (Fig. 2). We propose that this increase results from the forward shift of the estradiol surge into the time frame when the blood was withdrawn at 6 PM and is not only the result of an enhanced synthesis of estradiol. If this assumption is correct, then by examining the daily 6 PM samples for the initiation of the rise in estradiol in Fig. 2 the approximate time of the forward shift in the estradiol surge relative to delivery can be identified. Such a surveillance of our data suggests the forward shift in the estradiol surge occurs about 10 to 12 days before delivery.

The important implication of the forward shift in the nocturnal estradiol surge is that it provides a means of increasing the ratio of estradiol to progesterone without having a dramatic change in the synthesis or catabolism of these two steroid hormones. Thus we are hypothesizing that unlike nonprimate species, where the change in estradiol/progesterone ratio is achieved by decreasing progesterone and increasing estradiol production near term, a simple forward shift in the nocturnal estradiol surge results in an asynchronous 24hour rhythm of progesterone and estradiol. This shift provides a daily 3 to 5 hour window of elevated estradiol/progesterone ratio that is sufficient to induce molecular messages that initiate nocturnal uterine activity and ultimately lead to labor and delivery. This hypoth-

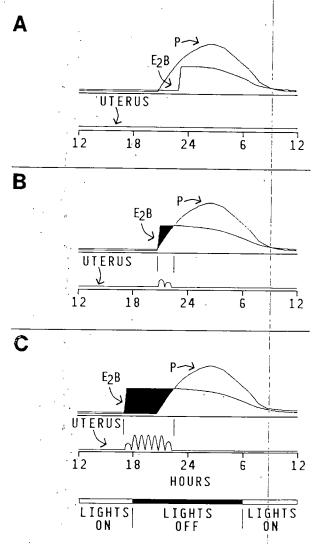


Fig. 8. Diagrammatic representation of hypothesis on initiation of nocturnal uterine activity. A, Early in third trimester progesterone (P) and estradiol ($E_2\beta$) show significant 24-hour rhythm with nocturnal estradiol surge following progesterone surge. With advancing gestational age (B and C) initiation of estradiol surge shifts forward and precedes progesterone surge. Coincident with estradiol shift is development of nocturnal uterine contractions. We propose that this forward shift in estradiol surge not only induces biochemical changes that result in uterine contractions but also initiates molecular messages that are the genesis for labor and delivery in the baboon (hours refer to 24-hour clock).

esis is summarized in Fig. 8. Another point to make is that the nocturnal activity is finite; that is, as the estradiol surge shifts forward and initiates uterine contractions, these contractions eventually terminate. We are proposing that the nocturnal uterine contractions are transient because of the nocturnal progesterone surge that blocks estradiol action at a critical threshold. Limited nocturnal uterine activity begins when the estradiol surge has shifted only slightly forward of the progesterone surge; thus the window of elevated estradiol and progesterone is small, and uterine contractions last for only a short period of time. As gestational age advances, the window becomes larger and the contractile activity lasts longer.

The hypothesis suggests that the surge in estradiol either prompts an immediate (within 1 hour) release of oxytocin and/or an increase in uterine oxytocin receptors or induces changes that are expressed 24 hours later. If estradiol action is immediate, it must not be acting by the classical genomic pathway for receptor action, which should take hours to initiate events that result in expression of cellular messages. Recent data suggest that progesterone may act nongenomically,30,31 and perhaps estradiol has similar effects. Catechol estrogens act on cell membranes similar to the way catecholamines do. Estradiol can be converted to catechol estrogens at the level of the hypothalamus32 and uterus,33 and perhaps it can enhance oxytocin release or increase the number of uterine oxytocin receptors. The second possibility is that estradiol acts genomically, and the response we observed results from an estradiol surge from the preceding 24 hours. Initial observations in our laboratory (unpublished observations) support the latter explanation. Also, this might explain why all animals did not show a one-to-one relationship between the shift in the estradiol surge and uterine contractions.

The cause of the forward shift in the nocturnal estradiol surge is not known. However, the literature provides a basis for at least a partial explanation. The fetal adrenal gland secretes increasing amounts of the estradiol precursor dehydroepiandrosterone sulfate with advancing gestation, particularly during the last 10 to 14 days. 18, 34 The fetal adrenals are under the control of corticotropin and other factors such as prolactin and growth factors.35 Fetal adrenal dehydroepiandrosterone sulfate secretion surges nocturnally; this correlates with nocturnal uterine activity.18 Cortisol or dexamethasone administered to pregnant humans or rhesus monkeys can eliminate nocturnal uterine activity and lower estradiol production by crossing the placenta and inhibiting fetal corticotropin release.36-38 In the pregnant rhesus monkey,18 human,39 and baboon6 maternal cortisol shows a 24-hour rhythm, attaining a peak around 6 to 10 AM. It has been proposed that as maternal cortisol decreases during the day, the negative feedback effect of cortisol on the fetal hypothalamicpituitary system diminishes, which in turn results in enhanced fetal corticotropin release and increased fetal dehydroepiandrosterone sulfate secretion and ultimately elevated placental estradiol production. Thus the fetal adrenal secretion of dehydroepiandrosterone sulfate would be 180 degrees out of phase with maternal cortisol.18 Advancement in the timing of the daily fetal adrenal activation could be due to a number of factors. A decrease in sensitivity of the fetal hypothalamic-pituitary axis to the negative feedback effects of maternal cortisol is one potential explanation. 40, 41 A second possibility is that adrenal secretagogues, such as prolactin (decidual or pituitary), or corticotropin secretagogues, such as placental corticotropin-releasing hormone or vasopressin, increase with advancing gestational age and enhance adrenal dehydroepiandrosterone production.35 Once the estradiol shift has begun, it could then increase the placental conversion of cortisol to cortisone, a biologically inactive corticosteroid. This has been demonstrated in the baboon placenta. 55 The decrease in maternal cortisol entering the fetus would further promote the advance in the nocturnal estradiol surge. Although this is only a hypothesis, it provides a rationale for the phenomenon of the forward shift in the initiation of the nocturnal estradiol surge. Additional studies will be required to definitively explain the forward shift in the estradiol surge.

The hypothesis suggests that the heightened estradiol/progesterone ratio induces uterine oxytocin receptor formation and increases production, or lowers the threshold of reflex activation for release, of hypothalamic oxytocin. However, the timing of oxytocin release is dependent on higher centers that are influenced by the light/dark cycles. 22, 28 Thus the increase in the estradiol/progesterone ratio is not only permissive in allowing oxytocin to be released and act on the uterus but perhaps obligatory for oxytocin synthesis. This is supported by observations in rhesus monkeys.26 In these studies fetal death was associated with a loss of nocturnal uterine activity coincident with diminished maternal estradiol levels. However, daily administration of estradiol benzoate by intramuscular injection to the mother reestablished the nocturnal uterine activity. Therefore this suggests that the purpose of the shift in the estradiol surge is to remove it from the inhibitory influence of progesterone, but the actual timing of the uterine activity is determined by higher centers that are influenced by the light/dark cycle. This hypothesis would also explain why the expression of the enhanced ratio of estradiol to progesterone takes about 24 hours. The increase in the estradiol/progesterone ratio only "sets the table" while the higher centers that are controlled by the light/dark cycle establish the precise timing of, and are the driving force for, the 24-hour rhythm of uterine activity. This part of the hypothesis is not supported by the human studies that report the loss of the estriol rhythm at term²⁰ but enhanced nocturnal uterine activity.42 Humans may be uniquely different in this respect, or the 24-hour rhythms in maternal estriol may still be present but not detectable in peripheral blood because of factors described above.

In summary, during the last trimester of pregnancy in the baboon the levels of estradiol and progesterone show a 24-hour rhythm, reaching baselines around 12 AM to 4 PM hours and surging nocturnally between 7 PM and 10 PM. As term approaches, the initiation of the nocturnal surge in estradiol shifts forward so that it precedes the progesterone surge. This shift in estradiol coincides with the beginning of nocturnal uterine activity. Since the nocturnal activity can be inhibited by an oxytocin antagonist, this suggests that oxytocin is a primary regulator of uterine contractions. Thus the surge in estradiol could either enhance oxytocin release, increase uterine sensitivity to oxytocin, or both. The precise timing of the oxytocin release is under the influence of higher brain centers. On the basis of these data we hypothesize that the forward shift in the initiation of the nocturnal surge of estradiol creates a daily window of increased estradiol/progesterone ratio that induces biochemical alterations (e.g., an increase in uterine oxytocin receptors or release of oxytocin) that result in nocturnal uterine activity and generate molecular messages that are the genesis for labor and delivery in the baboon.

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Measurement of human epidermal growth factor receptor in the endometrium during the menstrual cycle

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To evaluate a potential physiologic role of the epidermal growth factor receptor in the endometrium, we measured the receptor content at different times in the menstrual cycle. Endometrial biopsy specimens were obtained from 28 normal women during the proliferative or secretory phase of the menstrual cycle, and the epidermal growth factor receptor content was determined. When the number of epidermal growth factor binding sites were evaluated as a function of time within each phase, a difference between phases became evident. The level of receptor increased during the proliferative phase with a maximum just before ovulation (p = 0.0128, r = 0.748). The epidermal growth factor receptor level decreased during the secretory phase, reaching a minimum before menses (p = 0.0001, r = 0.843). We conclude that the endometrial epidermal growth factor receptor content is cycle dependent, being maximal during the periovulatory period and minimal just before or during menses. These findings further suggest a physiologic role for epidermal growth factor in the proliferation and differentiation of the endometrium. (AM J OBSTET GYNECOL 1991;165:1499-503.)

Key words: Epidermal growth factor receptor, endometrium, menstrual cycle

Epidermal growth factor (EGF), a 53-amino-acid single-chain polypeptide, is a potent cellular mitogen for a variety of cell types.1 Studies of EGF administration in vivo or in vitro have been shown to result in accelerated proliferation and differentiation of a multitude of tissues of ectodermal origin.2 In vitro EGF administration results in increased cellular protein, ribonucleic acid and deoxyribonucleic acid synthesis, and enhanced cell multiplication.2 EGF exerts its biologic activity through interaction with specific transmembrane receptors, which have been found to be overexpressed in various human malignant tumors, such as squamous cell carcinomas of the head, neck, lung, and skin,3 and tumors of the thyroid,4 brain,5 breast,6 bladder,7 and colon.8 The presence of these abnormally elevated numbers of EGF receptors have been associated with more aggressive tumors and a poor prognosis. 6.7 This overexpression of the EGF receptor sugests a disrup-

tion of the normal regulatory mechanisms of cellular growth in which cell proliferation is controlled by regulation of the EGF receptor in the tissue.

The EGF receptor was identified in the human myometrium, endometrium, and leiomyomas. These findings support a role for EGF and its receptor in the proliferation and differentiation of the endometrium during the normal menstrual cycle. Research in this area has produced conflicting results, with no agreement as to the expression of the EGF receptor in the endometrium as a function of the menstrual cycle. EGF receptor content has been reported as follows: (1) being not different between the proliferative and secretory phases of the menstrual cycle, 10, 11 (2) being higher in the proliferative than the secretory phase, 12 and (3) exhibiting cyclic variation during the menstrual cycle.

We performed endometrial biopsies in 28 women of reproductive age at documented time points in the proliferative or secretory phase of the menstrual cycle. The endometrial tissues were assayed for the presence of the EGF receptor to investigate its expression in endometrium and establish a correlation for EGF receptor expression during the proliferative and secretory phases of the menstrual cycle.

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Material and methods

Materials. Chloramine-T and Coomassie Brilliant Blue G were purchased from Sigma Chemical Company, St. Louis; minimum essential medium was obtained from Hazelton Biologics, Inc., Lenexa, Kan.; bovine serum albumin (fraction 5) was purchased from Gibco Laboratories, Grand Island, N.Y.; recombinant human EGF was supplied by Upstate Biotechnology,

Inc., Lake Placid, N.Y., and carrier-free sodium-iodine 125 was obtained from E.I. Dupont de Nemours & Co., Wilmington, Del.

Patients. The study population consisted of women of reproductive age undergoing diagnostic laparoscopy or laparoscopic tubal sterilization at our ambulatory surgery center. The human use committee of our institution approved the research protocol before patient recruitment started. The participants were recruited from the infertility and family planning services at Walter Reed Army Medical Center, Washington, D.C. They were taking no medications, and their menstrual histories were normal. Patients with amenorrhea, abnormal menstrual cycles, abnormal uterine bleeding, recent use of oral contraceptives (within the last 4 months), pelvic inflammatory disease, or pregnancy were excluded from this study. Before the scheduled surgery the research protocol was explained to all candidates, and informed consent was obtained from participants. On the day of the scheduled laparoscopic procedures, endometrial biopsies were performed with a Novak curette while the patients were under anesthesia. The biopsy specimens were taken in an uniform manner from the anterior, posterior, and lateral walls of the uterine fundus. A representative sample of each endometrial biopsy specimen was forwarded to our gynecologic pathologist for histologic dating, and the reminder of the biopsy specimens were immediately frozen in liquid nitrogen. The endometrial tissue was stored at -70° C until assayed. All participants were contacted within 2 to 4 weeks of surgery to document the date of onset of the next menstrual cycle. This information, together with the histologic results, was used to date the endometrial tissue.

Preparation of murine EGF and iodination of human EGF. Pure murine EGF was isolated by the method of Savage and Cohen.14 Recombinant human EGF was radioiodinated by the chloramine-T technique with carrier-free sodium-125I.15 The specific activity of the radiolabeled human EGF was adjusted to 1.5×10^6 counts/min/ng by the addition of nonlabeled human EGF.

Preparation of endometrial membranes. Endometrial tissues were homogenized in five volumes of icecold 10 mmol/L Tris hydrochloride, ph 7.5, containing 25 mmol/L sucrose and 1 mmol/L ethylenediaminetetraacetic acid with 15 slow strokes in a 7 ml Dounce homogenizer. The homogenates were centrifuged in a Beckman JA-20 rotor at 800 g at 4° C for 10 minutes. Nuclear pellets were discarded, and the resultant clear supernatants were centrifuged in a Beckman 60Ti rotor at 120,000 g at 4° C for 60 minutes. Supernatants were discarded and the resultant endometrial membrane pellets were resuspended in minimal essential medium containing 0.2% bovine serum albumin. The protein content of the resuspended membrane pellets was determined by the Bradford method,16 and the final endometrial membrane protein concentration was adjusted to 6 mg/ml.

Radioreceptor assay. This assay is based on the observation that nonlabeled EGF competes in an equimolar basis with radiolabeled EGF for the plasma membrane receptor and the fact that both murine and human EGF elicit nearly identical biologic responses.1,2

Binding assays were conducted in a total volume of 200 µl in 1.5 ml polyallomer microcentrifuge tubes. In all assays, 25 µl (150 µg) of endometrial membrane suspension was added to each tube. The binding reaction was initiated by the addition of 125I human EGF at nine separate concentrations ranging from 0.125 to 35 ng/ml to the microcentrifuge tubes. The incubations were performed in duplicate at room temperature for 90 minutes with gentle shaking. After incubation, 1 ml of ice-cold minimal essential medium containing 0.2% bovine serum albumin was added to each tube, followed by vortex blending. The tubes were immediately centrifuged in a Surespin centrifuge at 9500 g for 5 minutes. The supernatants were aspirated and the bound radioactivity in the pellets was counted in an LKB 1274 automatic γ -counter with a counting efficiency of 73%.

Specific binding was obtained from the differences between total and nonspecific binding that was determined in parallel in the presence of a 500-fold excess of nonlabeled murine EGF. Nonspecific binding accounted for 20% of the total binding. Scatchard¹⁷ plot analysis of the specific binding was performed for each endometrial membrane preparation, the number of binding sites, and the dissociation constant were determined. Statistical analysis of the data was performed by (1) the two-sample t test for independent samples, when the binding data of the proliferative and secretory phases were compared with each other and (2) simple linear regression, for evaluation of the binding data within each phase of the menstrual cycle.

Results

The participants in the study had a mean age of 30.2 years, with a range of 25 to 38 years. A total of 28 endometrial samples were obtained from these patients, 10 of which were in the proliferative phase and 18 in the secretory phase of the menstrual cycle. In Table I the participants in the study are divided according to the phase of menstrual cycle, and their characteristics are presented. The EGF radioreceptor assay was performed in all of these samples, and the EGF receptor content and dissociation constants were calculated for each endometrial sample and for each phase of the menstrual cycle. The binding of 125I human EGF to the endometrial membrane preparations was specific, because it was largely abolished by excess nonla-

Table I. Characteristics of the participants in the endometrial EGF binding study

Characteristic	Follicular phase* $(n = 10)$	Secretory phase* $(n = 18)$	Combined phases $(N = 28)$
Age (yr)	30.3	30.11	30.19 ± 4.13
Gravidity	2.0	1.5	1.68 ± 1.96
Parity	1.1	0.68	0.82 ± 1.28
Abortion	0.5	0.6	0.2 ± 0.63
Elective termination of pregnancy	0.4	0.78	0.64 ± 1.22
Cycle length (days)	30.0	27.39	28.32 ± 4.23

^{*}Mean values.

Table II. Binding characteristics in menstrual cycle

Characteristic	Proliferative phase $(n = 10)$	Secretory phase $(n = 18)$	p Value*	
K _d (mol/L)				
Mean	8.80×10^{-10}	8.34×10^{-10}	0.74	
SEM	3.10×10^{-10}	3.80×10^{-10}		
EGF binding sites (fmol/mg of protein)				
Mean	24.56	24.31	0.96	
SEM	8.73	15.31	'	

^{*}Results from comparison of proliferative and secretory phases with two-sample t test for independent samples.

beled murine EGF. The specific binding of 125 I human EGF also was saturable, with similar concentrations needed to attain saturation within each phase of the menstrual cycle. The calculated within-assay and between-assay variabilities for the EGF binding assay were 9.17% and 10.47%, respectively.

In the proliferative phase endometrium, the mean (±SEM) dissociation constant calculated from the individual K_d values obtained from the Scatchard plots was $8.80 \pm 3.10 \times 10^{-10}$ mol/L. The mean (\pm SEM) total number of EGF binding sites present in the endometrial membranes, as calculated from the binding data from each endometrial sample, was 24.56 ± 8.73 fmol/mg of protein, with a minimum and maximum number of 15.84 and 39.52 fmol/mg of protein, respectively. In the secretory phase endometrium, the mean (±SEM) dissociation constant calculated was $8.34 \pm 3.80 \times 10^{-10}$ mol/L, and the mean total number of available EGF binding sites was 24.31 ± 15.31 fmol/mg of protein, with a minimum and maximum number of 3.43 and 58.69 fmol/mg of protein, respectively. The mean K_d and mean number of EGF binding sites available in the membrane preparations from each phase of the menstrual cycle were compared with each other by means of the two-sample t test for independent samples. No significant differences were found between the proliferative and secretory phases in mean K_d values and mean number of EGF binding sites, with p = 0.74 and 0.96, respectively. These data are presented in Table II.

However, when the number of EGF binding sites was

evaluated as a function of time within each phase of the menstrual cycle, a difference between proliferative and secretory phases was evident. With advancing proliferative phase an increase in number of EGF binding sites was observed, which was maximal before the expected time of ovulation. Simple linear regression, performed with the EGF receptor content (number of EGF binding sites) of the endometrial membranes as the dependent variable and the proliferative phase day in which the sample was obtained as the independent variable, revealed a significant positive linear correlation with r = 0.748 and p = 0.0128 (Fig. 1). In the secretory phase the number of EGF binding sites was maximal at the beginning, decreasing with advancing secretory phase, being minimal before the time of expected menses. Simple linear regression performed with the EGF receptor content as the dependent variable and the secretory phase day as the independent variable revealed a very highly significant negative linear correlation with r = 0.843 and p = 0.0001 (Fig. 2). No significant linear correlation was seen between the dissociation constant and phase day in the proliferative and secretory phases of the menstrual cycle, with p = 0.99and 0.8, respectively.

Comment

Our study demonstrates the cyclic variation in endometrial EGF receptor content during the menstrual cycle as a function of time, confirming the previous report by Taketani and Mizuno.18 In addition, our data are in agreement with reports by Sheets et al.10 and

[†]Mean values ± SEM.

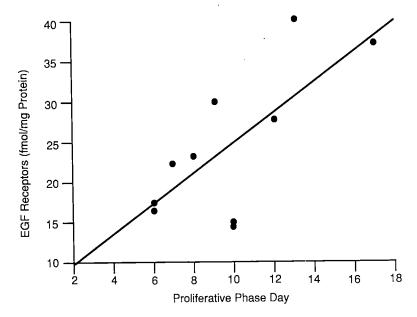


Fig. 1. Total number of EGF receptors as function of proliferative phase. Number of receptors is equal to number of binding sites as determined in Methods section. Significant positive linear correlation between EGF binding and advancing proliferative phase day was seen, p = 0.0128, r = 0.748.

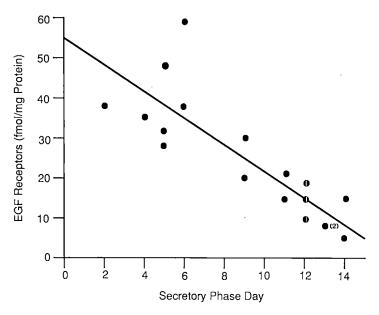


Fig. 2. Total number of EGF receptors as function of secretory phase. Number of receptors is equal to number of binding sites as determined in Methods section. Significant negative linear correlation between EGF binding and advancing secretory phase day was seen, p = 0.0001, r = 0.843.

Chegini et al.,11 in which mean EGF receptor contents of each phase of the menstrual cycle were found to be similar. Recently, Bonaccorsi et al. 12 published their results, which are in disagreement with these findings. They reported higher EGF receptor expression in endometrium of the proliferative as compared with the secretory phase. They used pooled endometrial membranes from 11 samples of proliferative and eight sam-

ples of secretory endometrium in their binding assays. To explain their results, we can only postulate that the endometrial samples were obtained late in the proliferative and secretory phases of the menstrual cycles. Then, on the basis of our findings, we would expect a higher receptor expression in the proliferative phase as compared with the secretory phase; these results are comparable to their findings.

During the menstrual cycle, the endometrium is characterized by cyclic changes of cell proliferation, differentiation, and death in response to sex steroids of ovarian origin. Although the exact mechanisms by which these changes take place are unknown, evidence is accumulating that implicates the regulation of growth factors and their respective receptors by sex steroid hormones. Most of the evidence being gathered originates from animal models. With these models, EGF and its receptor have been identified in uterine homogenates¹⁸ and uterine membrane preparations,¹⁹ respectively. In addition, the in vivo and in vitro effects of EGF and 17β-estradiol have been extensively studied. Administration of EGF, but not 17\u03c3-estradiol, to uterine epithelial cells in culture significantly stimulates cellular proliferation in a concentration-dependent manner.26 In vivo, 17β-estradiol administration results in an increase in uterine EGF receptor expression,21 which is preceded by an increase in uterine EGF receptor messenger ribonucleic acid.22

A hypothesis has been suggested to explain some of mitogenic effects of estrogen, in which estrogen promotes growth by regulating the levels of certain growth factors, such as EGF, or their receptors.²² Since estrogen action is the end result of binding to its high-affinity nuclear receptor, we can theorize that changes in their concentrations may affect the expression of EGF and its receptor in endometrium. During the menstrual cycle, as the estradiol concentration and estrogen receptor expression increase during the proliferative phase, a parallel increase in EGF receptor is seen. This increase is maximal during the periovulatory period, when the estradiol concentration and estrogen receptor expression also are at their maximum. After ovulation, progesterone secretion will result in a decrease in total estrogen receptor content and an increased endometrial metabolism of estradiol.23 As a result, even in the presence of high estradiol levels, with advancing secretory phase the EGF receptor content will decrease. This theory of estrogen regulation of EGF receptor expression in endometrium explains the cyclic variation in EGF receptor content seen during the normal menstrual cycle.

In summary, we have demonstrated the cyclic variation in EGF receptor expression during the menstrual cycle as a function of time and presented a possible mechanism by which estrogen and its receptor may regulate EGF receptor expression during the menstrual cycle. Although our findings give support to this theory of estrogen regulation of endometrial EGF receptor content, further studies are needed to elucidate the precise molecular mechanisms by which estrogens may regulate EGF receptor expression in endometrium.

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Fetal cardiovascular and fluid responses to maternal volume loading with lactated Ringer's or hypotonic solution

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To determine whether elevations in maternal vascular pressures or reductions in maternal osmolality would promote fluid transfer to the fetus, we intravenously infused either lactated Ringer's solution or diluted (hypotonic) lactated Ringer's solution continuously over 4 hours into late-gestation pregnant sheep. During the Ringer's solution infusion, the increases in maternal arterial (20.7 ± 1.7 mm Hg, mean ± SE) and venous (6.6 \pm 0.9 mm Hg) pressures were significantly greater (ρ < 0.00001) than those during the hypotonic infusion (6.6 \pm 1.5 and 1.7 \pm 0.6 mm Hg, respectively). The maternal osmolality changes during the Ringer's infusion (-5.7 ± 1.2 mOsm/kg at 1 hour and $+6.8 \pm 1.1$ mOsm/kg at 5 hours) were significantly less (p < 0.00001) than those during the hypotonic infusion (-10.2 ± 1.1 mOsm/kg at 1 hour and -15.9 ± 2.5 mOsm/kg at 5 hours). Fetal vascular pressures and blood volume were unchanged during either infusion. Fetal heart rate decreased by 15 to 20 beats/min by 1.5 hours of infusion in both groups but remained decreased only in the hypotonic group. Fetal urine flow decreased at the end of the Ringer's infusion and increased during the hypotonic infusion. These urine flow changes correlated with opposite changes in fetal plasma osmolality. The four-quadrant amniotic fluid index tended to increase in both groups, with an overall nonsignificant increase of 32% ± 16% 1 hour after the infusions. In summary, our findings suggest that (1) acute increases in maternal vascular pressures do not appear to promote fluid transfer to the ovine fetus and (2) acute decreases in maternal osmolality result in a small shift of fluid into the fetus as evidenced by an increase in fetal urine flow. (AM J OBSTET GYNECOL 1991;165:1504-15.)

Key words: Fetus, blood volume, urine flow, osmolality

Although it is clear that the human fetus accumulates 2 to 2.5 l of fluid from its mother during normal gestation, little is known to date about the mechanisms controlling this fluid transfer. If maternal factors are involved, from theory, the force(s) responsible could be either osmotic or hydrostatic pressure. There presently exists no clear understanding of whether physiologic alterations in one or both of these factors in the mother would lead to changes in fetal fluid balance. Previous animal and human studies have suggested that each may play a role. In terms of osmolality, it has been shown in animals and humans that induced increases or decreases in maternal osmolality are accompanied by similar changes in fetal osmolality. These osmolality changes in the fetus have been accepted to reflect

alterations in fetal fluid status even though (1) fetal volume changes have not been measured during reductions in maternal osmolality and (2) ovine fetal blood volume decreased only transiently during acute elevations in maternal osmolality and returned to normal within 1 hour. Furthermore, when pregnant ewes were subjected to several days of dehydration, no change from normal in fetal blood volume was found. Thus it is unclear whether fetal volume status actually varies with alterations in maternal osmolality within the physiologic range, although an extreme elevation in maternal osmolality has been shown to reduce the fluid volume of fetal rabbits.

The effects of variations in maternal hydrostatic pressures are similarly unclear. Animal studies have found no fetal fluid responses when isotonic fluid was administered to the mother.^{3,8} However, these previous studies are problematic because only small volumes were administered and maternal vascular pressures may have been unchanged. On the other hand, Goodlin et al.⁹ reported that pregnant women who were believed to have hypovolemia responded to oral or intravenous volume expansion with an increase in amniotic fluid volume. A recent case report by Sherer et al.¹⁰ gives futher credence to the suggestion that maternal volume expansion may increase fluid flux to the fetus. In that report a patient who presented with significant hypovolemia and severe oligohydramnios responded to

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vigorous volume resuscitation (6500 ml fluid) with a normalization of the maternal volume status and amniotic fluid volume. Although the latter two studies suggest a role for maternal volume effects on the fetus, it is unclear whether changes in maternal vascular pressures or changes in osmolality provided the driving force for fluid transfer to the fetus, particularly in view of the fact that the maternal plasma sodium fell from 141 to 136 mEq/L in the latter study, suggesting that a reduction in osmolality may have been involved.

Because these observations do not provide a clear understanding of the effects of acute alterations in maternal hydrostatic or osmotic forces on fetal fluid dynamics, we evaluated whether fluid infused into pregnant sheep in sufficient quantities to elevate maternal venous pressure or reduce maternal blood osmolality would affect fetal fluid status. As indices of the latter, we determined fetal blood volume and composition, vascular pressures, urine output, and the four-quadrant amniotic fluid index.

Methods

Animal preparation. Seven gravid ewes with a gestational age (mean \pm SE) of 124 \pm 1 day at the time of surgery were used in this study. We followed university rules and regulations and United States Public Health Service guidelines for the care and use of laboratory animals. This protocol was approved by our institution's animal subjects committee.

With animals under gas inhalation anesthesia and with sterile surgical techniques, polyvinyl catheters were inserted in the fetal femoral arteries and veins, amniotic cavity, and urinary bladder as previously described.4,11,12 Polyvinyl catheters also were placed in the maternal inferior vena cava through a femoral vein and in the abdominal aorta through both femoral arteries for measurement of venous and arterial pressure and for blood sampling. A large-bore catheter was placed in the maternal inferior vena cava through the contralateral femoral vein for fluid infusion. The animals were maintained on a regimen of prophylactic antibiotics for the first 5 postsurgical days with daily flushing of the catheters with a heparin solution.12

Protocol. The first experiment was conducted 5 to 7 days after catheter placement while the ewe was maintained in a metabolic cart. In a random fashion, on days separated by at least 48 hours, the animals were infused intravenously with either lactated Ringer's solution (Baxter) or with lactated Ringer's solution diluted with sterile water to a final osmolality of 150 mOsm/kg. The experimental protocol consisted of a 1-hour control period, a 4-hour continuous infusion period, and a 1hour recovery period. All animals were subjected to at least one Ringer's and one hypotonic infusion. In the animals subjected to more than one Ringer's or hypotonic infusion, the volume of the infusate was purposely varied to explore a range of responses. A total of eight Ringer's and 11 hypotonic infusions was performed.

Experimental design. The objective of this study was to determine whether alterations in maternal hydrostatic or osmotic forces would promote fluid transfer to the fetus. For the hydrostatic portion of the study, we infused sufficient lactated Ringer's solution to elevate maternal venous pressure by 5 to 10 mm Hg. For the osmotic portion of the study, we infused sufficient hypotonic Ringer's solution (150 mOsm/kg) to reduce maternal osmolality by 3% to 10%. We refer to these as the Ringer's infusion and the hypotonic infusion, respectively. The rationale for infusing lactated Ringer's solution is that it is readily available and widely used. Although it is not exactly isotonic to normal blood (sodium, 130 mEq/L; potassium, 4 mEq/L; calcium, 3 mEq/L; chloride, 109 mEq/L; lactate, 28 mmol/L; measured osmolality, 255 mOsm/kg), we anticipated that the actual changes in maternal osmolality would be small. For the hypotonic infusion, the rationale for dilution of the Ringer's solution to 150 mOsm/kg was that this is the lowest osmolality that can be rapidly infused into the circulation and not cause extensive hemolysis.

Sampling and analytic measurements. At 30-minute intervals during the control period and 1-hour intervals thereafter, samples of maternal and fetal blood and urine and amniotic fluid were taken. The fetal blood samples (3 ml) were replaced with an equal volume of heparinized maternal blood drawn immediately before study. Electrolytes and osmolality were measured in all samples, and hematocrit and plasma protein concentration were determined in all blood samples. Fetal blood volume as a percent of that during the control period was calculated from fetal hematocrit with a correction for osmotically induced changes in red blood cell volume as previously described.4 This correction is important because hematocrit would be expected to increase when osmolality decreases, even though blood volume remains unchanged. Using this method, a 1% change in fetal blood volume can be detected with a 99% degree of confidence.4 The concentrations of arginine vasopressin and atrial natriuretic factor in fetal plasma samples were measured as previously described.13 Throughout the control, infusion, and recovery periods, fetal and maternal arterial and venous pressures and heart rates plus amniotic fluid pressure and fetal urine flow were recorded continuously on a polygraph recorder, digitized, and stored on disk at 30second intervals with an on-line computer as previously described.11 Fetal vascular pressures were corrected continuously with amniotic fluid pressure used as the zero pressure with the on-line computer The four1506 Powers and Brace November 1991
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Table I. Fetal, maternal, amniotic, and urinary osmolalities and concentrations during 1-hour control period

Variable	Fetal blood	Maternal blood	Amniotic fluid	Fetal urine
Osmolality (mOsm/kg) Sodium (mEq/L) Chloride (mEq/L)	298.2 ± 0.9 141.1 ± 0.4 109.4 ± 0.3	300.9 ± 0.9 144.4 ± 0.5 113.5 ± 0.3	274.1 ± 9.1 115.1 ± 5.5 95.0 ± 4.3	228 ± 24 43.2 ± 5.1 41.3 ± 4.0
Potassium (mEq/L)	4.0 ± 0.1	4.4 ± 0.1	6.6 ± 1.0	11.7 ± 4.4

Values are mean ± SE.

Table II. Fetal values during 1-hour control period

Variable	Mean ± SE
Arterial pressure (mm Hg)	47.1 ± 1.1
Venous pressure (mm Hg)	3.8 ± 0.5
Heart rate (beats/min)	172 ± 3
Urine flow (ml/min)	0.47 ± 0.11
Arterial pH	7.336 ± 0.005
Carbon dioxide tension (mm Hg)	$54.5~\pm~0.5$
Oxygen tension (mm Hg)	21.0 ± 0.6
Hematocrit (%)	35.7 ± 0.9
Plasma protein (gm/dl)	4.0 ± 0.1
Amniotic fluid index (mm)	79.3 ± 11.7
Plasma arginine vaso- pressin (pg/ml)	3.9 ± 0.6
Plasma atrial natriuretic factor (pg/ml)	156 ± 13

quadrant amniotic fluid index was measured during the control and recovery periods as previously described. He Briefly, the uterus was divided into four quadrants that were scanned by ultrasonography. The amniotic fluid index equaled the sum of the largest vertical pocket of fluid in each of the four quadrants determined in triplicate.

Necropsy. After the experiments were completed, the animals were killed with a euthanasia solution (pentobarbital sodium, 300 mg/kg) and fetal weight was recorded.

Data presentation and statistical analysis. The data are presented as means \pm SEs. In some of the figures, the data are plotted as the mean \pm SE change from the average value during the control period.

We used an unpaired t test to compare values during the control period in the Ringer's infused compared with the hypotonic-infused animals. Because there were no statistically significant differences between the two groups, they were combined.

To determine whether there were statistically significant changes with time in each group, we used a two-factor repeated measures analysis of variance, with time and animal being the fwo factors. For comparing the Ringer's versus the hypotonic groups, we used a three-

factor analysis of variance, with time, treatment, and animal being the three factors. The interaction term from the latter analysis of variance was used to determine whether the changes in time in the two groups were statistically different. For exploring relationships among variables, bivariate and multivariate linear regression techniques were used. The symbols r and R are used to designate the bivariate and multivariate correlation coefficients, respectively. For the regression analyses, we used mean values during the control period and means during the last 1 hour of each infusion, expressed as changes from preinfusion values. Significance was taken as $p \le 0.05$ and described changes are significant at the $p \le 0.05$ level, unless specified otherwise.

During the data analysis, we found that the fetal urine flow rate response to the Ringer's or hypotonic infusion depended on the urine flow rate during the control period. Thus we arbitrarily chose to subdivide our experiments into those with basal fetal urine flows either above or below 0.5 ml/min. Fetuses with a urine flow rate <0.5 ml/min are referred to as the low-flow group and those >0.5 ml/min as the high-flow group. Normal urine flow rate for fetuses at the same gestational age in this laboratory averages approximately 0.6 ml/min.¹⁵

Results

Fetal gestational age on the day of experimentation in the Ringer's (130 \pm 2 days) and hypotonic groups (135 \pm 2 days) was not significantly different. Mean fetal and maternal values during the control period are shown in Tables I and II. These values are consistent with normal values from this laboratory.

During the control period, several variables were statistically related. Fetal osmolality correlated positively with maternal osmolality (r=0.855, $p<10^{-5}$). Fetal urine flow rate was positively related to fetal arterial pressure (r=-0.624, p=0.0045). Finally, amniotic fluid osmolality (r=0.620, p=0.0046) and urine osmolality (r=-0.603, p=0.0063) were negatively related to fetal urine flow rate.

During the volume loading with Ringer's solution to elevate maternal venous pressure, we infused 5.55 ± 0.50 L/hr for 4 hours into the maternal vein.

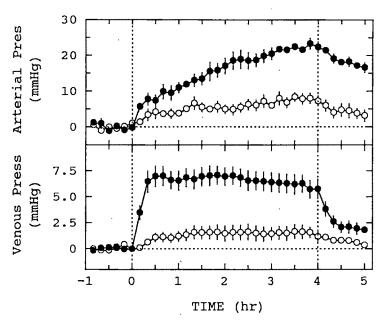


Fig. 1. Maternal arterial and venous pressure responses to Ringer's (dots) or hypotonic (circles) infusion into circulation of ewe. Data expressed as mean \pm SE change from average value during 1-hour preinfusion period. Vertical dotted lines show infusions started at 0 hour and ended at 4 hours.

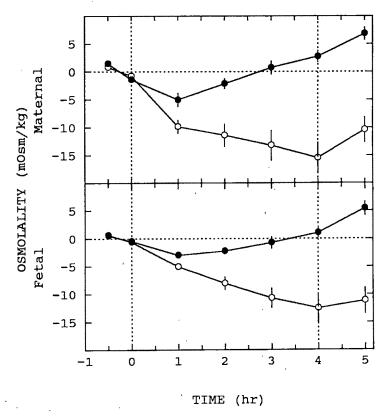


Fig. 2. Maternal and fetal osmolality changes during Ringer's (dots) or hypotonic (circles) infusion into vein of ewe. Data expressed as mean \pm SE change from average value during 1-hour preinfusion period. Vertical dotted lines show infusions started at 0 hour and ended at 4 hours.

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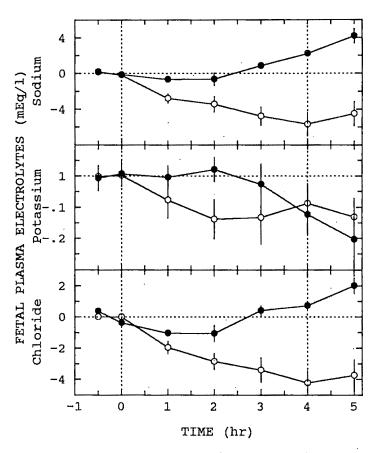


Fig. 3. Fetal plasma electrolyte concentration changes during Ringer's (dots) or hypotonic (circles) infusion into ewe's circulation. Data expressed as mean ± SE change from average value during 1-hour preinfusion period. Vertical dotted lines show infusions started at 0 hour and ended at 4 hours.

During the hypotonic infusion, 1.98 ± 0.29 L/hr were administered. The resulting changes in maternal arterial and venous pressures for the two different infusates are shown in Fig. 1. Maternal arterial and venous pressures were significantly elevated during both infusions (p < 0.00001) and the elevations were significantly greater during the lactated Ringer's infusion versus the hypotonic infusion (p < 0.0001). During the last 2 hours of the infusions, arterial and venous pressures were elevated by an average of 20.7 ± 1.7 and 6.6 ± 0.9 mm Hg, repectively, during the Ringer's infusion compared with 6.6 ± 1.5 and 1.7 ± 9.6 mm Hg during the hypotonic infusion.

Fig. 2. shows the infusion-induced changes in maternal osmolality. There were highly significant changes with time in both groups (p < 0.00001) and the decreases with the hypotonic infusion were significantly greater (p < 0.00001). Contrary to our expectations of a small, maintained decrease in osmolality over time in the lactated Ringer's group, maternal osmolality gradually increased after the initial decrease and was significantly above control at the end of the recovery period (p < 0.001). In the hypotonic group, maternal osmolality was decreased by $10.2 \pm 1.1 \text{ mOsm/kg}$ at 1

hour and gradually decreased further to 15.9 ± 2.5 mOsm/kg below control by the end of the infusion.

The fetal osmolality responses (Fig. 2) were highly significant (p < 0.00001) and paralleled the maternal responses (r = 0.946, p < 0.00001, during the last hour of infusion) but were slightly delayed and less in magnitude compared to maternal responses (analysis of variance interaction, p = 0.049 for hypotonic and p = 0.077 for Ringer's when comparing the fetal vs maternal osmólality changes). During Ringer's infusion, fetal osmolality initially decreased, followed by a gradual rise above preinfusion values at the end of the recovery period. In contrast, fetal osmolality decreased during the hypotonic infusion and remained reduced. These changes in fetal osmolality with time were significantly different (p < 0.00001) in the two groups. Fetal plasma electrolyte concentrations largely paralleled the changes in osmolality, as seen in Fig. 3. Fetal arterial pH increased by 0.03 units during the Ringer's infusion and was unchanged during the hypotonic infusion. Fetal carbon dioxide tension was unchanged during either infusion, whereas fetal oxygen tension was decreased only during the recovery period by an average of 1.8 mm Hg in both groups.

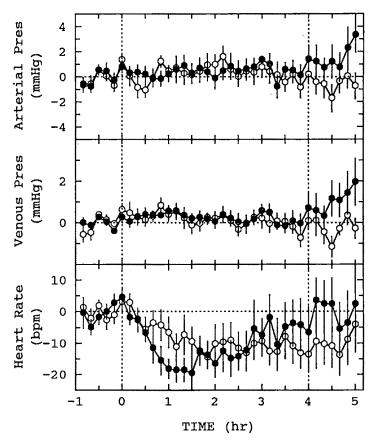


Fig. 4. Fetal vascular pressure and heart rate responses to Ringer's (dols) or hypotonic (circles) infusion into ewe. Data expressed as mean ± SE change from average value during 1-hour preinfusion period. Vertical dotted lines show infusions started at 0 hour and ended at 4 hours.

Fetal arterial and venous pressures were unchanged during either infusion, but there was a tendency for arterial and venous pressures to rise during the recovery period after Ringer's infusion (Fig. 4). Fetal heart rate initially decreased (p < 0.00001) by 15 to 20 beats/min in both groups (Fig. 4). During Ringer's infusion, fetal heart rate returned to control levels by the end of the infusion but remained reduced during the hypotonic infusion. These differences were highly significant (p = 0.0071). As seen in Fig. 5, fetal hematocrit decreased at the end of the 4-hour Ringer's infusion compared with an increase during the hypotonic infusion (p = 0.00020). Neither fetal plasma protein concentration nor the volume of blood circulating in the fetus underwent significant changes with time in either group (Fig. 5).

The fetal plasma arginine vasopressin and atrial natriuretic factor responses to the infusions depended on the infusate. As seen in Fig. 6, arginine vasopressin was unchanged during the Ringer's infusion, compared with a decrease averaging 20.9% ± 4.2% during the hypotonic infusion. Fetal plasma arginine vasopressin concentration increased above control in both groups at the end of the recovery period, and the overall

changes in arginine vasopressin with time in the combined groups were highly significant (p = 0.0004). Fetal plasma atrial natriuretic factor was unchanged during the hypotonic infusion (Fig. 6). Atrial natriuretic factor decreased by an average of 20 pg/ml during the isotonic infusion, but this was of marginal significance (p = 0.059).

Fetal urine flow rate underwent an increase during the hypotonic infusion; this was significantly different from the small increase, followed by a decrease, in urine flow rate during Ringer's infusion (p < 0.0001, Fig. 7). On closer inspection, we found that the fetal urine flow responses depended on the initial urine flow rate. That is, 2 hours after starting the Ringer's infusion, urine flow rate decreased in the fetuses with a high control flow, compared with no change with time in those fetuses with a low urine flow during the control period. Similarly, during the hypotonic infusion, fetal urine flow did not change in the animals with a high basal urine flow, but it increased significantly in the fetuses with a low basal urinary output. The changes in fetal urine flow during the last hour of the infusions correlated positively with the changes in fetal arterial pressure (p = 0.086), positively with the changes in fetal 1510 Powers and Brace November 1991
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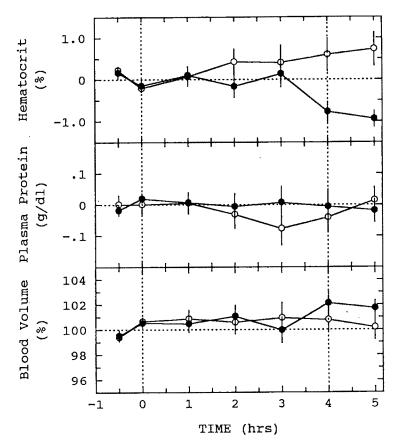


Fig. 5. Fetal hematocrit, plasma protein concentration, and blood volume responses to Ringer's (dots) or hypotonic (circles) infusion into the ewe. Data expressed as mean \pm SE change from average value during 1-hour preinfusion period for hematocrit and plasma protein concentration and as percent of control for blood volume. Infusions started at 0 hour and ended at 4 hours.

atrial natriuretic factor (p = 0.0053), and negatively with the changes in fetal blood osmolality (p = 0.0034), as determined by multivariate regression (R = 0.744; p = 0.006), but were not correlated simultaneously with the other variables.

Fetal urine composition was significantly altered during the infusions (Fig. 8). Fetal urine osmolality underwent a small transient decrease midway through the Ringer's infusion, followed by a rise above control levels. This was different (p < 0.00001) from the large decrease in urine osmolality during the hypotonic infusion. The changes in urine osmolality during the last hour of the infusion were inversely related to changes in urine flow (r = 0.865; p < 0.00001). Urine sodium and chloride changes paralleled the changes in urine osmolality, and differences in sodium and chloride between the two groups were marginally significant (p = 0.077 and p = 0.058 for Na⁺ and Cl⁻, respectively). There was no significant difference in fetal urine potassium between the two groups (p = 0.12).

Amniotic fluid composition underwent significant changes with time. Amniotic osmolality (Fig. 9) decreased with time (p = 0.0080), and the decrease in

the hypotonic group was only marginally different (p=0.111) from that during Ringer's infusion. Amniotic sodium and chloride concentrations paralleled the osmolality changes in that there were significant decreases with time (p=0.0121 and p=0.0105 for sodium and chloride, respectively), but the decreases during hypotonic infusion were not significantly greater than during Ringer's infusion. Amniotic potassium concentration did not change with time during Ringer's infusion but decreased during the hypotonic infusion. The amniotic fluid index tended to increase in both groups, with an overall increase of $32\% \pm 16\%$ I hour after terminating the infusions. These were not significant changes.

Comment

To better understand the extent to which acute alterations in maternal hydrostatic or osmotic forces may affect fetal fluid balance, we monitored the changes in fetal fluid and electrolyte status during short-term maternal volume loading in the pregnant ewe. We anticipated that either increasing the hydrostatic pressure or decreasing the osmotic pressure with volume loading

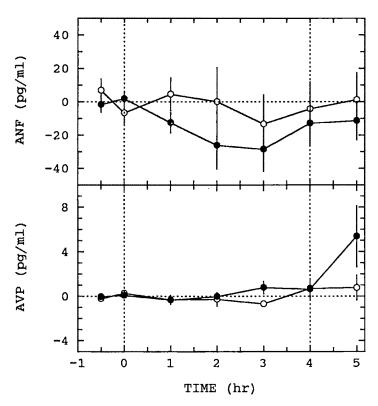


Fig. 6. Fetal plasma arginine vasopressin and atrial natriuretic factor responses to Ringer's (dots) or hypotonic (circles) infusion. Data expressed as mean ± SE change from average value during 1-hour control period.

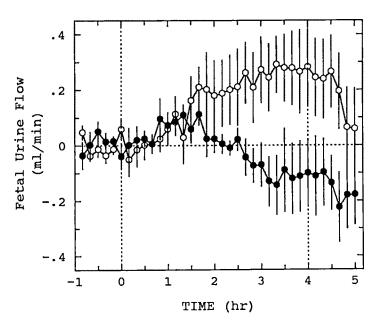


Fig. 7. Fetal urine flow rate responses to Ringer's (dots) or hypotonic (circles) infusion into ewe. Data expressed as mean ± SE change from average value during 1-hour preinfusion period. Infusions started at 0 hour and ended at 4 hours.

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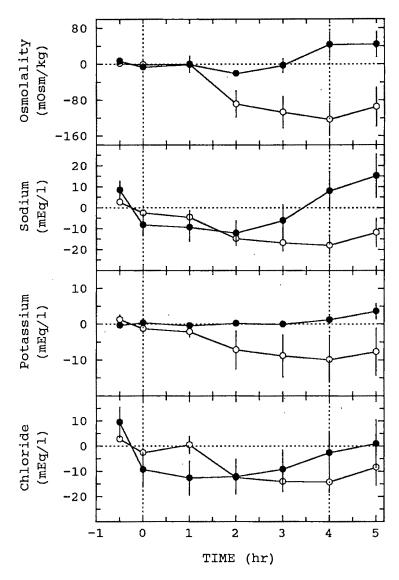


Fig. 8. Fetal urine compositional responses to Ringer's (dots) or hypotonic (circles) infusion. Data expressed as mean ± SE change from average value during 1-hour preinfusion period. Vertical dotted lines show infusions started at 0 hour and ended at 4 hours.

would result in significant force across the placenta to push fluid down a pressure gradient into the fetus. However, in spite of increases in maternal arterial and venous pressures of 20.7 and 6.6 mm Hg, respectively, during the Ringer's infusion, we found no increase in fetal arterial or venous pressures, blood volume, or urine flow. Collectively, these observations do not support the hypothesis that an increase in maternal vascular pressures will cause a significant transfer of fluid to the ovine fetus over a 4-hour period. It may be suggested that this is not surprising in view of the low value of the filtration coefficient that has been reported for the sheep placenta.16 However, in a recent study,17 we found a significatly higher value of the placental filtration coefficient such that at least 160 ml (i.e., 0.1 ml/min/mm Hg × 6.6 mm Hg × 240 minutes) may

have been expected to cross the placenta because of the changes in the hydrostatic pressure during our 4-hour infusion. In addition, it has also been shown that, when ovine fetuses were infused intravenously with 7 L of isotonic fluid over 3 days, five of the 7 L infused were transferred to the mother in the absence of any change in the fetal plasma composition.18 Thus it was surprising to see that a transfer of fluid to the fetal compartment was not evident during the Ringer's infusion. Furthermore, our observation of an actual decrease in fetal urine flow late in the infusion was unexpected but was consistent with theoretic considerations of the simultaneous increase in maternal osmolality. This late decrease in fetal urine flow suggests that either the concomitant rise in maternal osmolality had removed fluid from the fetus or that the associated rise in fetal os-

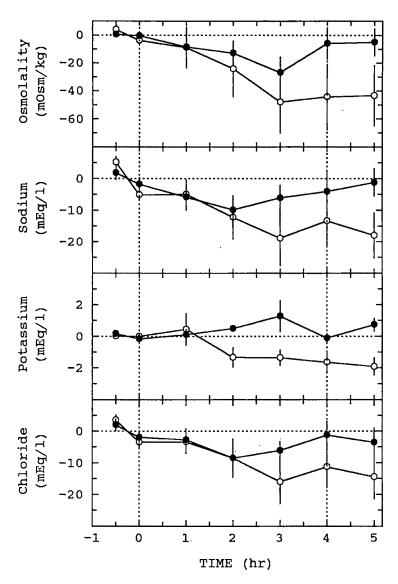


Fig. 9. Amniotic fluid compositional responses to Ringer's (dots) or hypotonic (circles) infusion. Data expressed as mean \pm SE change from average value during 1-hour preinfusion. Vertical dotted lines show infusions started at 0 hour and ended at 4 hours.

molality stimulated a late rise in fetal arginine vaso-pressin, which produced an antidiuretic effect. The latter mechanism is consistent with the observed changes in fetal arginine vasopressin concentrations and with the lack of change in fetal blood volume. This mechanism is also consistent with prior observations during maternal dehydration in sheep. ¹⁹⁻²¹ This reemphasizes the fact that the changes in plasma osmotic pressure are potentially much more powerful than vascular hydrostatic pressure changes because each 1 mOsm/kg change in osmolality can produce up to 19.7 mm Hg of osmotic pressure change at 39.5° C. Thus the late increase in maternal osmolality appears to have determined the net fetal fluid response to the Ringer's infusion. However, if this were the case, a transient in-

crease in fetal fluid status would have been expected after 1 to 2 hours of Ringer's infusion when the changes in maternal hydrostatic and osmotic pressure both favored fluid transfer to the fetus. In fact, there was a slight but not statistically significant increase in fetal urine flow at that time. This suggests that there may have been a transient increase in fetal blood volume causing the fetal diuresis.

Unlike the Ringer's infusion, the hypotonic infusion resulted in significant increases in fetal urine flow when maternal plasma osmolality was lowered by as little as 3% to 5%. However, the increase in fetal urine flow was delayed relative to the decrease in maternal plasma osmolality such that, for the first hour of the infusion, fetal urine flow did not change despite a significant

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drop in maternal plasma osmolality to 10 mOsm/kg below control. Thereafter, there was a gradual increase in fetal urine flow over the second hour of the infusion. This finding is consistent with prior studies in waterloaded adults22 where peak water diuresis was delayed for 90 to 120 min, the time required for clearance of arginine vasopressin from the extracellular space. The decrease in fetal plasma arginine vasopressin concentration in this study was similarly delayed, suggesting that the delayed rise in fetal urine flow was due to a delayed clearance of arginine vasopressin from the fetal circulation. Furthermore, the decrease in urine osmolality paralleled the decrease in fetal plasma arginine vasopressin. Thus the changes in fetal urine flow and osmolality are consistent with the observed changes in fetal plasma arginine vasopressin concentrations.

When the hypotonic experiments were separated into those where the animals began with a low urine flow during the control period versus those with an initial high flow, there was a significant difference in the response to a lowering of maternal osmolality. In animals where the fetus had an initial low urine flow, there was a significant increase in urine output with hypotonic infusion, whereas there was no increase in urine output in those animals with a high urine flow during the control period, in spite of similar decreases in maternal and fetal osmolality. This suggests that, when fetal urine flow is high and plasma arginine vasopressin concentration is low, arginine vasopressin does not affect the fetal kidney. Thus any further reduction in fetal arginine vasopressin level during hypoosmolality does not alter fetal urine flow or osmolality.

The alterations in fetal urine osmolality, electrolyte concentrations, and flow certainly suggest fluid moved into the fetus during the hypotonic infusion, and fluid was withdrawn from the fetus late during the Ringer's infusion. However, this could not be documented by a statistical change in fetal vascular pressures, blood volume, or amniotic fluid index. This apparent discrepancy can be explained by the fact that only about a 100 to 200 ml change in fetal fluid volume was expected during the 4-hour infusions. Futhermore, previous studies have found that little fluid was retained intravascularly in the fetus after short-term²⁸ or chronic¹⁸ intravascular infusions. Thus the lack of change in fetal blood volume is not inconsistent with the concept that fluid was transferred into the fetus during the hypotonic infusions and out of the fetus late during the Ringer's infusion when maternal plasma osmolality was elevated. This does not diminish the importance of maternal vascular or osmotic pressure changes in overall fetal fluid balance, because the volume of fluid which may have crossed the placenta would result in an enormous fluid flux over the course of gestation. Long-term studies will be necessary to document changes in parameters other than fetal urine flow.

In summary, our study failed to support the hypothesis that acute increases in maternal vascular pressures would promote fluid transer to the ovine fetus. Instead, the fetal fluid response to maternal volume loading appeared to be determined by the changes in maternal osmolality, with decreases in maternal osmolality producing an increase in fetal urine flow and vice versa. Over a period of days, these changes could potentially significantly alter fetal fluid balance.

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Oxytocin secretion and human parturition: Pulse frequency and duration increase during spontaneous labor in women

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The secretory pattern of oxytocin was determined in blood samples taken at 1-minute intervals for 30 minutes from 32 parturient women. The samples were collected in a manner that minimized degradation by plasma oxytocinase, and a highly specific antibody was used for the radioimmunoassay. The results indicated that oxytocin is secreted in discrete pulses of short duration. The frequency of the pulses was significantly higher during spontaneous labor than before the onset of labor. The mean pulse frequencies per 30 minutes were 1.2 ± 0.54 before labor, 4.2 ± 0.45 during the first stage, and 6.7 ± 0.49 during the second and third stages of labor. The mean pulse durations in these three groups were 1.2 ± 0.20 , 1.9 ± 0.28 , and 2.0 ± 0.26 minutes, respectively. The amplitude of the pulses was variable with no significant differences between the groups, the majority being around $1.0~\mu\text{U/ml}$. The spontaneous pulses were of similar magnitude as those measured in 18 women after intravenous injections of 4 to 16 mU of oxytocin, which doses stimulated uterine contractions. We therefore conclude that the pulses of oxytocin observed at increasing frequency during spontaneous labor are of physiologic significance and provide evidence for the participation of oxytocin in the onset and maintenance of spontaneous labor. (AM J OBSTET GYNECOL 1991;165:1515-23.)

Key words: Oxytocin, pulsatile secretion, spontaneous labor, women, onset of labor

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In spite of clear evidence for increased secretion of oxytocin during parturition in animals, changes in maternal oxytocin secretion rates during labor in women remain a matter of controversy. Both the absolute levels in peripheral circulation and the temporal variations in the course of labor vary widely in the published reports. Some authors found maternal oxytocin levels to remain unchanged throughout late pregnancy and labor, others found increasing levels in late pregnancy but decreasing levels during labor, and others observed a rise in the second stage only. In our laboratory we consistently found maternal levels to be

slightly but significantly raised over term—no labor values. Using a bioassay, Coch et al. found maternal levels in the "jugular" vein (obtained through a catheter pushed cranially into the subclavian vein) to be increased during labor with maximal levels in the second stage, whereas samples of peripheral plasma obtained at the same time showed no increase. Characteristic for all studies, whether performed by radioimmunoassay (RIA) or bioassay, is the great variability between individual values, even in patients not in labor. In fact, Chard and Gibbens reported their results in terms of the percentage of samples that showed values above the detection limit; they found that this percentage increased in the course of labor and concluded that their findings might indicate spurt release.

Dawood et al.⁶ reported widely fluctuating minuteto-minute variations in six pregnant women, three of whom were in labor. The authors interpreted these fluctuations as pulses, although they did not show pulse characteristics and may have represented the random variability of their assay.

The measurement of circulating oxytocin in pregnant women is difficult because of its slow plasma concentration, which in turn is a consequence of the extreme potency of oxytocin to stimulate the uterus at term. On the basis of oxytocin infusion studies, the effective concentrations at term are estimated to be in the range of 1 to 10 pmol/L. Another factor contributing to the difficulties is the presence of the enzyme oxytocinase (L-cystine-aminopeptidase) in high concentrations in pregnancy plasma of primates. This enzyme rapidly degrades the endogenous oxytocin present in the blood samples after they have been withdrawn, unless special precautions are taken. Moreover, the degradation products may share epitopes with the intact molecule and therefore may cross react with some of the antibodies used in the current RIAs. This may explain the differences in the absolute oxytocin levels reported in the literature.

If oxytocin were secreted in a pulsatile manner in women as it is in cows¹ and sheep,¹⁰ the measurement of oxytocin secretion rates is further complicated. The wide fluctuations in maternal plasma levels observed in most studies are compatible with a pulsatile pattern of release, but the sampling frequency has not been sufficient to demonstrate a pulsatile pattern in the previous studies nor were any of the studies subjected to rigorous analysis of the data to exclude random variations due to extraction and RIA procedures. The purpose of this study was to reexamine the secretory pattern of oxytocin in pregnant women before and during labor with a highly specific and sensitive antibody and a high sampling frequency, together with the application of precise criteria for pulse identification.

Material and methods

Subjects. Fifty pregnant women (38 to 42 weeks of gestation) without any serious medical or obstetric complications participated in this study, which was approved by the institutional ethics boards and human rights committees at both institutions concerned in the patient selection (Yale—New Haven Hospital and Hospital Sotero del Rio). All patients gave consent to have serial samples of blood taken during a 30-minute period. Uterine activity was monitored in all patients by means of external cardiotocography (Advanced Medical Technology, Humden, Conn.).

Sample collection. Samples (5 ml each) were collected with 1-minute intervals for 30 minutes from the following three groups of women (150 ml total volume from each woman): (1) women at term scheduled for elective cesarean section who had a closed cervix and were not in labor (n = 11), (2) women in the first stage of spontaneous labor with <6 cm cervical dilatation (range, 2.5 to 5.5 cm; mean \pm SD, 4.5 \pm 1.17 cm; n = 13), (3) women in the second stage of spontaneous labor with full dilatation of the cervix (n = 8). Five of the women of group 3 were delivered during the sampling period. Samples continued to be taken until the delivery of the placenta, and the sampling period in these patients therefore includes both the second and third stages. An additional fourth group of pregnant women at term was included (n = 18). They were not in labor and were given a bolus injection of oxytocin intravenously; the dose given was 2, 4, 8, or 16 mU. Most women had only one dose each; a few were given another dose 15 to 30 minutes after the first dose. Eight blood samples of 5 ml were collected in each instance (total, 40 ml) beginning immediately before and 30 seconds after, and 1, 2, 3, 4, 5, and 10 minutes after the intravenous injection.

The samples were collected from an indwelling heparin-lock catheter into precooled tubes containing an excess of ethylenediaminetetraacetic acid (0.37 mol/L) to inhibit the activity of oxytocinase. We did not add phenanthroline because in the dose recommended it caused hemolysis. The tubes were immediately put into an ice bath and kept on ice at all times. They were centrifuged at 4° C as soon as possible and plasma was immediately separated, acidified to pH 2 with hydrochloric acid (1N, 0.2 ml/ml plasma), and frozen until assayed. The low pH inhibits oxytocinase activity.

Oxytocin assay. Two milliliters plasma was used for each assay. The acidified plasma was diluted with an equal volume of 0.2N hydrochloric acid and passed through a column containing 500 mg heat-activated Florișil (Sigma, St. Louis). Oxytocin was eluted from the column with 90% acetone in water; the extracts were dried and then dissolved in 250 µl of assay buffer.

Standards in the range from 0.125 to 5 µU/ml were added to charcoal-treated pregnancy plasma and extracted in the same way as the unknowns with each assay to determine extraction recovery. The antibody used was a gift from Dr. Mariana Morris, Department of Physiology, Bowman Gray School of Medicine, Winston Salem. It is highly specific and sensitive and does not, in contrast to our old oxytocin antibody,12 cross react with the estrogen-induced peptide that was first described by Amico et al.13 The antibody has been validated for use in pregnant primates14 and pregnant women.15 Separation of bound and free ligand was by means of Pansorbin cells (Calbiochem, San Diego). During these studies the detection limit was 0.1 to 0.2 μU/ml (mean, 0.17 μU/ml) after correction for extraction recovery, which was $65.4\% \pm 8.7\%$ (mean \pm SD). The mean intraassay coefficient of variation was 11.3% and the interassay coefficient of variation was 15.7%. All samples from one patient were run in the same assay. The standards were from the National Bureau of Standards, London, which performs the standardization by bioassay and expresses the oxytocin concentrations in international units, approximately 1.68 µg peptide per 1 IU.

Plasma oxytocinase activity determinations. One sample from each patient was used for measuring Lcystine aminopeptidase activity. S-Benzyl-L-cysteine-βnitroanilide was used as a substrate.16 Before assay, the sample was neutralized to pH 7.4 and an excess of Ca++ (10 mmol/ml) was added to reactivate the enzyme. All chemicals were from Sigma.

Data analysis. The RIA results were calculated after logit-log transformation of the data with a computerized program (National Institutes of Health RIA logit),17 which also calculates the coefficient of variation at each level of standards and unknowns. Pulse analysis was performed according to the computerized method (Pulsar) of Merriam and Wachter. 18, 19 A calibration data set for the selection of cutoff criteria was created by analyzing three pools of pregnancy plasma in 30 duplicate samples each. One pool was charcoal treated, to represent baseline, one had 0.75 μ U/ml, and the third 1.5 μ U/ml added. The cutoff criteria selected were 3 × SD for single peaks and $2.5 \times SD$ for broader peaks. This minimized spurious peaks (0% in the baseline pool) and maximized the detection of peak levels in the other two pools. Mean peak pulse oxytocin level and mean overall oxytocin level were calculated for each patient (for nondetectable values the detection limit of the assay was used). The means for individual patients were used to calculate the means for each group; therefore n =number of patients. The values given are group means \pm SE, with n in parentheses. The pulse dura-

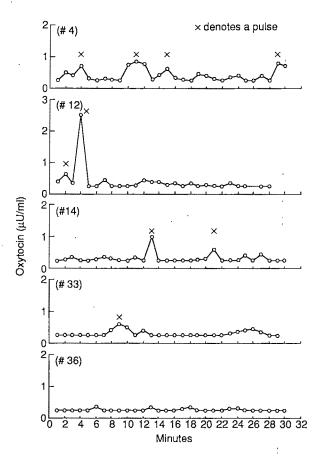


Fig. 1. Plasma oxytocin levels in five women in late pregnancy not in labor. Samples were collected at 1-minute intervals from indwelling catheter in right arm. Data plots in this and Figs. 2 and 3 were chosen to show the highest and lowest pulse frequencies and amplitudes in each group. Each identified pulse is denoted with cross. Six women of 11 in the no-labor group showed no pulses during study period.

tion was defined as the number of consecutive 1minute samples that the level was above the pulse limit.

Statistical analysis. One-way analysis of variance was used to compare the mean values for the three groups, followed by Dunnett's test. A value of p < 0.05 was considered significant; the Student t test was used when two groups were compared.

Results

The basal levels of oxytocin were below detection limit of the assay, 0.17 µU/ml, in all patients. In the 11 women not in labor (group 1), the percentage of samples below detection limit was 85%, it was 30% in group 2 (13 women in the first stage of labor), and it was 16% in group 3 (eight women in the second and third stages of labor). The mean pulse limit for single peaks was $0.45 \,\mu\text{U/ml}$ (baseline $\pm 3 \,\text{SD}$ at baseline), and all values exceeding this level were considered pulses. Discrete pulses of oxytocin were observed in all groups. They

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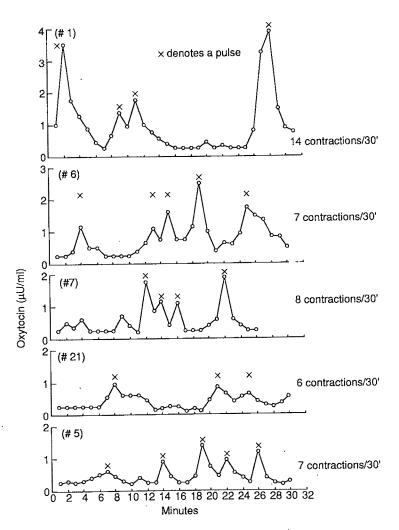


Fig. 2. Plasma oxytocin levels in five pregnant women during first stage of labor (cervix, 2 to 5 cm dilated). For details, see legend to Fig. 1. All women in this group had oxytocin pulses during study period.

occurred at irregular intervals and had variable amplitudes (peak levels). All were of short duration, 1 to 3 minutes. Thirty-minute plots of the data from several women in each group are shown in Figs. 1 through 3. The plots selected show the range of variability observed in each group, with the one showing the highest frequency and amplitude of pulses at the top and those showing the lowest pulse frequency and amplitude at the bottom of each figure (Figs. 1 through 3).

The frequency of oxytocin pulses was lowest in women not in labor (group 1), who had on the average 1.2 ± 0.54 pulses per 30 minutes; several of the women of this group (6 of 11) had no pulses during the study period. All women in labor, on the other hand, had oxytocin pulses; the mean frequency during the first stage of labor (group 2) was 4.2 ± 0.54 pulses per 30 minutes (range, 1 to 7 per 30 minutes), and during the second and third stages (group 3) the pulse frequency was 6.5 ± 0.49 pulses per 30 minutes (range, 4 to 8

per 30 minutes). The differences in pulse frequency were highly significant, p < 0.001 (Fig. 4).

The amplitude of the pulses (peak levels) ranged from 0.5μ to 8.2μ U/ml, but the majority were in the range of 0.5 to 1.5 µU/ml in all groups. The mean pulse amplitude in women not in labor (group 1), $0.91 \pm 0.20 \,\mu\text{U/ml}$ (n = 11), was lower than in women in labor, $1.10 \pm 0.10 \,\mu\text{U/ml}$ (n = 21) (groups 2 and 3 combined), but the difference was not significant. Women in the second and third stages of labor had the highest mean pulse amplitude, $1.40 \pm 0.35 \, \mu \text{U/ml}$ $(p < 0.05 \text{ vs group } 1, \alpha_1)$. The mean duration of the pulses was also increased in women in labor in comparison with those not in labor, 1.94 ± 0.21 versus 1.2 ± 0.14 minutes (groups 2 and 3 vs group 1, p < 0.01, α_2). As a result of the higher pulse frequency, amplitude, and duration the overall mean plasma levels of oxytocin were significantly higher in women in labor than in women not in labor (Table I).

Effect of exogenous oxytocin. Injection of oxytocin as an intravenous bolus resulted in a detectable rise in plasma oxytocin levels in all women except some of those receiving the lowest dose, 2 mU. Peak levels were usually observed at 30 seconds, in a few instances at 1 minute after the injection. The rise was dose dependent (Fig. 5). A significant rise was detected in two of six patients receiving the 2 mU dose and in all receiving the other doses, 4, 8, and 16 mU. Peak levels were similar to those observed during spontaneous pulses; the majority of spontaneous pulses resembled those produced by 4 and 8 mU injected intravenously.

The injections of oxytocin caused uterine contractions in the majority of women; in two of five receiving 2 mU; four of five receiving 4 mU, five of six receiving 8 mU, and one of two receiving 16 mU. A series of contractions leading to the onset of labor occurred in one woman after the injection of 8 mU of oxytocin. On the average, the number of contractions in the first 10 minutes after the injection was correlated with the mean peak plasma level in each group (Fig. 6), but the correlation in individual women between the peak plasma oxytocin level and the number of contractions recorded by external tocography was not significant.

Plasma oxytocinase. Plasma oxytocinase activity was present in all women (range, 25 to 105 U/L). The women in labor had higher oxytocinase activity than those not in labor, but the extraction recovery of oxytocin, which was measured in one sample from each individual patient, was not correlated with plasma oxytocinase activity, indicating that the rapid cooling and addition of ethylenediaminetetraacetic acid were effective in blocking the enzyme activity during sample preparation. In samples taken during infusions of 10 mU oxytocin per minute we found the levels to be similar (3 to 4 µU/ml) as those reported by Thornton et al.11 who used a combination of phenanthroline and ethylenediaminetetraacetic acid in concentrations that they claim to be 100% effective in preventing oxytocinase activity. Some inactivation of plasma oxytocin may of course have occurred while the blood was being drawn and before it became thoroughly mixed with the ethylenediaminetetraacetic acid in the tube and was cooled. Our data therefore may underestimate the true values somewhat.

Comment

Our results unequivocally demonstrate that the secretion of oxytocin is significantly increased in spontaneous labor in comparison with women at term but not in labor. The secretion occurs in short pulses, and the frequency of these pulses is significantly increased during labor. There is no absolutely right method to use in the analysis of episodic hormone secretion. We have chosen the method of Merriam and Wachter¹⁸. ¹⁹

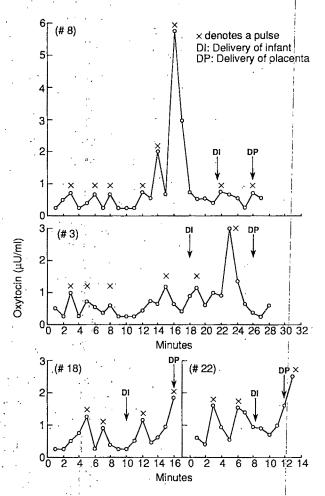


Fig. 3. Plasma oxytocin levels in three pregnant women during second and third stages of labor. *DI*, Delivery of infant; *DP*, delivery of placenta.

because it takes into account the assay variability at each point and rescales all identified peaks in terms of this unit, an important consideration that the levels are so low as plasma oxytocin. The cutoff values are based on the width of each peak, and the algorithm selects both narrow high peaks and broader peaks that may be lower. The program does not depend on pattern recognition, nor does it require a pattern in the timing of the peaks that may be inherently irregular, as is the case with oxytocin secretion. The choice of cutoff criteria is based on empiric considerations. Increasing the cutoff criteria for our data would have decreased the number of identified pulses but would not have changed the conclusion, namely, that the frequency of pulses was increased during labor in comparison with no-labor válues.

Of particular interest is the finding that oxytocin secretion is significantly increased in early labor, in our experimental subjects at cervical dilatations of 2 to 5 cm. We did not observe a marked and prolonged increase in plasma oxytocin during the third stage of

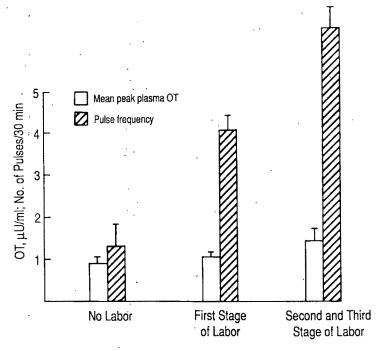


Fig. 4. Mean oxytocin pulse frequency and amplitude determined in women at term, not in labor (n = 11), during first stage of labor (n = 13), and combined second and third stages of labor (n = 8). Oxytocin concentrations were measured by RIA in samples collected at 1-minute intervals for 30 minutes in each group; values greater than baseline \pm 3 SD were considered a pulse. Means of all pulses in individual patient were calculated. Values given represent mean of these means \pm SE; n = 1 number of patients in each group.

Table I. Oxytocin secretion in pregnant and parturient women at term

Group	n	Pulse frequency (No./30 min)	Pulse duration (min)	Pulse amplitude (µU/ml)	Mean oxytocin over 30 min (μU/ml)	Cervical dilatation (cm)
Group 1. No labor	11	1.3 ± 0.54	1.2 ± 0.20	0.91 ± 0.20	0.26 ± 0.041	Closed
Group 2. First stage	13	$4.2 \pm 0.45*$	$1.9 \pm 0.28*$	0.92 ± 0.12	$0.42 \pm 0.04*$	4.5 ± 1.2
Group 3. Second and third stage	8	$6.5 \pm 0.49 \dagger$	2.0 ± 0.26*	1.4 ± 0.35	$0.65 \pm 0.12*$	10

Values are mean ± SE of means for individual patients; n, number of patients.

labor as seen by Thornton et al.11 in about 40% of patients.

The pulse amplitude during labor, although variable, was generally low, on the average, $1.1~\mu\text{U/ml}$, and not significantly greater than in women not in labor. Similar pulses were produced by injections of 4 to 16 mU of oxytocin. Since injections of these doses were effective in eliciting uterine contractions in women at term, the observed levels are of physiologic significance and are adequate for the activation of the myometrium at term and at the onset of spontaneous labor in particular, because of the prelabor rise in uterine oxytocin sensitivity to maximum values. ^{20, 21} Pulsatile oxytocin was found to be more effective per unit oxytocin than

continuous infusion in inducing uterine contractions and delivery in pregnant rats.²² Lower total doses were required with pulsed oxytocin than continuously infused oxytocin to induce labor in pregnant women.^{23, 24} In spite of higher intermittent pulse doses per minute than with the continuous infusion rates (mean maximum, 50 ± 4 mU/min every 10 minutes vs 20.2 ± 2.3 mU/min²³ and mean maximum, 41.6 ± 6 mU/min every 8 minutes vs 9.2 ± 1.8 mU/min²⁴), the total dose required was only 30% to 50% of the dose administered by continuous infusion. The pulsatile secretion pattern therefore provides more effective myometrial stimulation than does a constant tonic secretion.

^{*}Significant differences from values in women not in labor.

[†]Significant differences between values in first stage and second stage of labor.

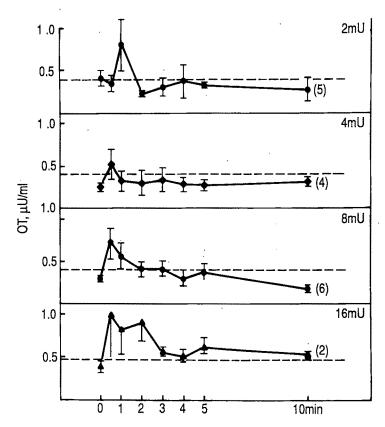


Fig. 5. Plasma oxytocin concentrations in women in late pregnancy measured before and after intravenous injection of synthetic oxytocin, in doses indicated in upper right corner of each panel. Values are mean; bars indicate SE; n is in parentheses. Dotted line, Mean pulse limit (cutoff criterion) for spontaneous pulses.

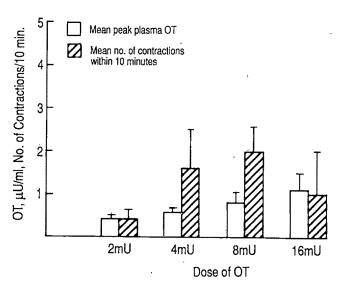


Fig. 6. Comparison of peak plasma oxytocin levels observed after intravenous injection of 2, 4, 8, and 16 mU oxytocin and number of contractions recorded by external cardiotocometer after each injection. Values are mean \pm SE; for n, see Fig. 2.

The fact that not every woman receiving an injection of oxytocin responded with contractions, although a given dose produced similar oxytocin levels in all, is in keeping with clinical observations of individual variations in uterine responsiveness to oxytocin near term. It is also in harmony with our previous findings regarding myometrial oxytocin receptor concentrations near term and during labor.20 Considerable individual 1522 Fuchs et al.

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variations in oxytocin receptor concentrations were present at 36 to 40 weeks of gestation before the onset of labor, but at the early stages of labor all women had significantly higher receptor concentrations than before the onset of labor. A good correlation was found between the rise in myometrial oxytocin receptor concentrations in early labor and the rise in uterine responsiveness to oxytocin in the last days of gestation.²¹

The factors responsible for the increased secretion of oxytocin in the early stages of labor are not known. Oxytocin is usually, but not always, secreted in response to neural stimuli. The uterine corpus and fundus are virtually depleted of neurotransmitters during pregnancy, and they are not replenished until sometime after delivery.25 Uterine innervation is therefore not likely to participate in the release of oxytocin at term. However, the innervation of the cervical region, which has a rich supply of both adrenergic and vasoactive intestinal polypeptidergic nerves, is retained unaltered during pregnancy25 and could play a role in the release mechanism of oxytocin at the onset of labor. In support of this notion is our finding that many women with cervical incompetence, particularly those who subsequently had preterm deliveries, have increased oxytocin levels in midpregnancy.15 The actual stimulus could be provided by pressure exerted by the descending fetus on the internal os from above or by the increasing tension to which the cervical region is subjected by the increasingly distended upper segment of the uterus. A reflex release of oxytocin in response to the distension of the cervix and the uterine body was postulated by Ferguson²⁶ in 1941 but its existence has so far not been convincingly demonstrated except in the ewe, goat, and rat.27-29 Fish et al.30 tried to cause oxytocin release by experimentally stretching the cervix in women in early labor with a balloon inserted inside the uterus in front of the amniotic sac. The balloon was inflated with 350 ml water, and strong traction was applied that dilated the cervix from 3 to 6 cm but did not cause milk ejection responses, as measured by simultaneous recording of intramammary pressure. Moreover, animal studies indicated that stimulation of organs in the deep pelvic cavity27 or vagina30 was more effective in eliciting oxytocin release than the manipulation and stretching of the cervix, and it is therefore possible that afferent pelvic nerves in various organs of the pelvis rather than in the cervix could be responsible for the release of oxytocin at the onset of labor in women. Vaginal distention may have elicited some of the increases in the late second stage and third stage reported by various authors.

In conclusion, we have shown that in pregnant women at term, oxytocin is secreted in short pulses of variable amplitude. During labor the secretion increases significantly. The increase involves mainly a rise in pulse frequency and to a lesser degree in pulse amplitude and duration. The spontaneous pulses are similar in magnitude as those produced by doses of oxytocin injected intravenously (4 to 16 mU) that are capable of eliciting uterine contractions in women at term. The spontaneous pulses of oxytocin therefore are likely to play a major role in uterine activation during spontaneous labor.

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Dose-dependent effects of angiotensin II on the ovine fetal cardiovascular system

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The purpose of this experiment was to establish dose-response relationships for the effects of angiotensin II on arterial pressure, venous pressure, heart rate, and blood volume in the ovine fetus. Chronically catheterized fetal sheep at 132 \pm 1 (SE) days' gestational age were infused with angiotensin II at 4.8 \pm 1.1 (n = 7), 27.7 \pm 4.6 (n = 7), 102.2 \pm 16.7 (n = 6), or 239.0 \pm 30.9 (n = 4) ng/min/kg fetal body weight for 30 minutes. Fetal arterial pressure increased at the three highest doses. Fetal venous pressure, heart rate, and blood volume responded only at 102.2 and 239.0 ng/min/kg. Åt 239.0 ng/min/kg, arterial pressure increased by 17.7 \pm 1.6 mm Hg (p < 0.00001), venous pressure increased by 1.5 \pm 0.3 mm Hg (p < 0.0005), blood volume decreased by 7.8 \pm 2.2% (p < 0.0001), and heart rate initially decreased by 14 \pm 4 beats/min, followed by an increase of 52 \pm 17 beats/min from control (p < 0.0005) at the end of angiotensin II infusion. Thus this study shows that angiotensin II affected multiple fetal cardiovascular variables in a dose-dependent manner, suggesting that it is an important regulatory hormone for the entire fetal cardiovascular system. (AM J OBSTET GYNECOL 1991;165:1524-33.)

Key words: Ovine fetus, arterial pressure, venous pressure, heart rate, blood volume, angiotensin II

Angiotensin II is a potent vasoconstrictive hormone in the fetus and the adult. The early work of Broughton-Pipkin et al.1 and others2-4 has shown that an active renin-angiotensin system is present in the ovine fetus as early as midgestation. Its major function appears to be maintenance of fetal arterial pressure under conditions of fetal stress, such as acute hemorrhage or hypoxia.1-3 Several investigators have examined the effects of exogenous angiotensin II in the ovine fetus. Previous studies have demonstrated a pressor effect on arterial pressure in the fetus.5-11 Clark et al.9 and Yoshimura et al.10, 11 reported a dose-response relationship for brief infusions (5 minutes) in the fetus. The doseresponse relationship for longer infusions has not been reported. In addition, the effect of angiotensin II on fetal heart rate (FHR) is unclear in that Iwamoto and Rudolph⁷ and Robillard et al.8 reported an increase in FHR after administration of angiotensin II to the fetus. Clark et al.9 and Yoshimura et al.10, 11 reported a decrease in FHR. Ismay et al.6 found that the heart rate response was dependent on fetal arterial pressure. Furthermore, the effects of angiotensin II on fetal venous pressure or blood volume have not been previously investigated. Because of these unknowns and contradictory effects of angiotensin II on FHR, we chose to examine in more detail its effects on the cardiovascular function of the fetus. We were particularly interested in determining whether it can modulate parameters of the fetal cardiovascular system in addition to arterial pressure. Thus our study was conducted to establish dose-response relationships in the near-term fetal sheep for the effects of angiotensin II on arterial pressure, venous pressure, heart rate, and blood volume.

Methods

Time-dated pregnant sheep were used for this study. The surgical preparation has been described in detail elsewhere.12 In brief, pregnant ewes with singleton fetuses at 126 ± 1 days' gestation were anesthetized with intravenous thiamylal sodium, intubated, and maintained on gas inhalation anesthesia (halothane, 1% to 2% in oxygen). A midline incision was made in the maternal abdomen, exposing the uterus. An incision was made in the uterus, and the hindlimbs of the fetus were exteriorized. Catheters were placed in a pedal artery and saphenous vein of each leg. Two additional catheters were attached to the fetal skin to measure amniotic fluid pressure. The catheters were exteriorized to a pouch sewn on the maternal flank. Postoperatively, the mother received penicillin G (600,000 U) and dihydrostreptomycin sulfate (750 mg) intramuscularly for 5 days, and ticarcillin disodium (500 mg) with clavulanate potassium (16 mg) was injected daily into the amniotic cavity for 5 days. Experimentation

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began on postoperative day five. This protocol was approved by our institution's animal subjects committee.

The experimental protocol consisted of a 30-minute control period and a 30-minute infusion of angiotensin II, followed by a 30-minute recovery period. Fetal arterial pressure, venous pressure, amniotic fluid pressure, and heart rate were continuously recorded on a polygraph recorder (Gould), and 30-second averages were stored on an on-line computer (Hewlett-Packard) as previously described.13 The vascular pressures were referenced to the standard zero pressure reference for the fetus by continuously subtracting amniotic fluid pressure with the on-line computer. At the lowest infusion rate the venous pressure could not be recorded in one fetus.

Before experimentation the pressure transducers were checked for zero drift over several hours and only transducers with minimal drift were used. Zero baseline pressure values were determined at the beginning of the experiment. On completion of each experiment baseline pressure correction was made for any drift that might have occurred during the course of experimentation, assuming that drift was linear with time. Typically the drift was small (<1 mm Hg) or nonexistent.

Angiotensin II diluted in isotonic saline solution was infused intravenously into the fetus at rates of 10 (n = 5), 30 (n = 4), 100 (n = 5), 300 (n = 5), or 1000 (n = 5) ng/min at a flow rate of 0.39 ml/min. The infusion catheter (volume equaled 1.25 ml) was primed with infusate (0.8 ml) 30 seconds before the angiotensin II infusion was started. We have previously shown that vehicle infusion does not alter any of the monitored variables.14 One to three experiments were performed on each animal with 48 hours between experiments. Infusion rates were randomly assigned, and no animal received the same infusion rate more than once. A total of 24 experiments were conducted in 15 animals.

Samples of 3 ml of fetal arterial blood were obtained every 15 minutes, starting 10 minutes after the beginning of the protocol. This was replaced immediately with an equal volume of heparinized maternal blood obtained before starting the experiment. The fetal blood was used for the measurement of pH, Pco2, and Po₂ (Instrument Laboratory System 1302 pH/blood gas analyzer at 39.5° C), hematocrit in triplicate,15 osmolality (freezing point depression with an Advanced Digimatic osmometer model 3D2), plasma protein concentration (American Optical, TS meter), and Na+, K+, and Cl⁻ ion concentrations (Nova 5+5 electrolyte analyzer). The change in fetal blood volume as a percent of control was calculated as the mean control hematocrit during the 30-minute preinfusion period divided by the hematocrit of each sample, as previously described.15

Angiotensin II concentration in the infusate was

Table I. Control values of fetus

·	· Mean ± SE*
Arterial pressure (mm Hg)	45.8 ± 1.0
Venous pressure (mm Hg)	4.1 ± 0.4
Heart rate (beats/min)	175 ± 4
Arterial pH	7.33 ± 0.01
Arterial Pco2 (mm Hg)	53.0 ± 0.5
Arterial Po ₂ (mm Hg)	20.8 ± 0.7
Hematocrit (%)	32.8 ± 1.2
Plasma protein (gm/dl)	3.8 ± 0.1
Plasma osmolality (mOsm/kg)	298 ± 1
Plasma sodium (mEq/L)	141.0 ± 0.3
Plasma potassium (mEq/L)	4.3 ± 0.1
Plasma chloride (mEq/L)	107.4 ± 0.4

^{*}N = 24, except N = 23 for venous pressure.

measured by a double-antibody radioimmunoassay as described previously16 with the following modification. The antiserum used for the assay was purchased from Peninsula Laboratory and was specific for human angiotensin II. The sensitivity of the assay was 0.3 pg, and the intraassay and interassay coefficients of variation were 4.0% and 3.4%, respectively. A 2 ml portion of each fetal blood sample was reserved for determination of angiotensin II concentration. However, our tests showed that the peptidase inhibitor (aprotinin) used in preserving the sample inadequately inhibited the degradation of angiotensin II. Therefore the results of the plasma angiotensin II assays were not accurate and could not be used. The protease inhibitor that we used differed from that used by Robillard et al.8 and Naden et al.,17 who used o-phenanthroline as the inhibitor in their plasma samples collected for angiotensin II measurements.

On completion of the experiment the animals were killed with an overdose of pentobarbital sodium solution. The fetuses were weighed after towel drying. Fetal weight on the day of experimentation was estimated, and a 3% increase in fetal body weight per day was assumed for fetuses not weighed on the day of experimentation.18 The weight-normalized infusion rates were determined from the measured infusate concentrations, and the fetal body weights were either obtained at autopsy or calculated as described above.

Data analysis. The infusion rates were divided into four groups on the basis of weight-normalized infusate concentrations measured by the assay: 4.8 ± 1.1 , 27.7 ± 4.6 , 102.2 ± 16.7 , or 239.0 ± 30.9 ng/min/kg fetal body weight. These groupings were selected to eliminate overlap of the infusion rates among the four

Data are expressed as the mean \pm SE. To determine whether changes with time in a variable were statistically significant, a two-factor analysis of variance for repeated measures was used, with animal and time being the two factors. Bivariate and polynomial regres-

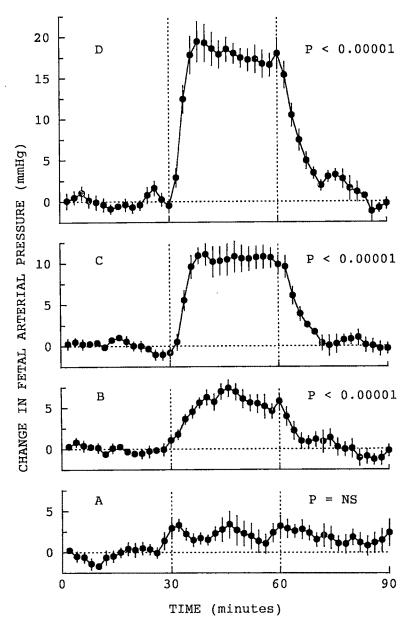


Fig. 1. Changes (mean \pm SE) in fetal arterial pressure during infusion of angiotensin II from 30 to 60 minutes. Data are expressed as change from control during 0 to 30 minutes. Infusion rates were 4.8 \pm 1.1 (A, n=7), 27.7 \pm 4.6 (B, n=7), 102.2 \pm 16.7 (C, n=6), and 239.0 \pm 30.9 ng/min/kg (D, n=4).

sion analysis was used to determine the relationship between infusion rate and response. Multivariate regression analysis was used to determine the relationships among several variables simultaneously. Results were considered statistically significant if $p \le 0.05$.

Results

Mean values for fetal arterial pressure, venous pressure, heart rate, arterial pH and blood gases, hematocrit, plasma protein, osmolality, Na^+ , K^+ , and Cl^- during the control period are shown in Table I. On the day of experimentation (n=24) the mean gestational

age of the fetuses was 132 ± 1 (SE) days. Mean fetal weight at the time of experimentation was 3.02 ± 0.17 kg.

Fetal arterial pressure increased significantly from baseline values during infusion of angiotensin II at all infusion rates except the lowest (p < 0.0001, Fig. 1). The rise in arterial pressure began quickly with the onset of infusion and reached a peak value within 4 minutes. The greatest increase in arterial pressure was 17.7 ± 1.6 mm Hg (p < 0.001); this occurred at the highest infusion rate. A decline in arterial pressure was observed within 2 minutes after the infusion was

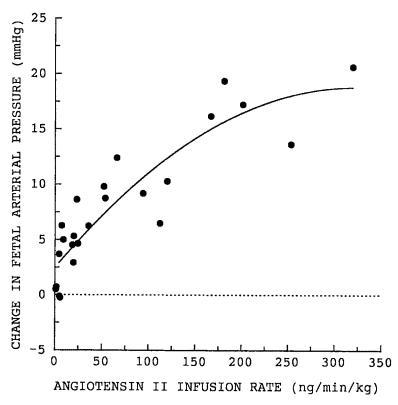


Fig. 2. Second-order polynomial regression plot of change in fetal arterial pressure averaged over last 20 minutes of angiotensin II infusion for each experiment (total of 24) versus infusion rate of angiotensin II (r = 0.90, p < 0.00001).

stopped. Polynomial regression analysis showed a significant correlation between the fetal arterial pressure response and infusion rate during the last 20 minutes of angiotensin II infusion (r = 0.90, p < 0.00001, Fig. 2).

Compared with the arterial pressure changes, the fetal venous pressure response to angiotensin II was less dramatic, with a significant increase occurring only at infusion rates of 102.2 ± 16.7 and 239.0 ± 30.9 ng/min/kg (p < 0.005, Fig. 3). At the lowest infusion rate there was an increase in venous pressure before the onset of the angiotensin II infusion. On review of the individual polygraph tracings, there was increased uterine activity and fetal movement occurring at this time, which probably accounts for this elevation in pressure. At $239.0 \pm 30.9 \text{ ng/min/kg}$, the peak increase in venous pressure was 1.5 ± 0.3 mm Hg. Similar to the arterial pressure response, the maximal increase in venous pressure occurred within 4 minutes of the onset of the infusion. With termination of infusion, venous pressure declined toward baseline within 1 to 2 minutes. Venous pressure declined below baseline during the recovery period at the two highest infusion rates, with a maximal decrease of 2.2 ± 0.7 mm Hg (p <0.05) at 239.0 \pm 30.9 ng/min/kg. At the highest infusion rate fetal venous pressure returned to baseline by the end of the recovery period. Linear regression analysis showed a significant relationship between the mean elevation in venous pressure during the last 20 minutes of the infusion and the infusion rate (r = 0.47, p < 0.05, Fig. 4). Analysis with polynomial regression did not produce a better-fitting regression line.

With the onset of angiotensin II infusion at 102.2 ± 16.7 and 239.0 ± 30.9 ng/min/kg, FHR decreased immediately. At an infusion rate of 102.2 ± 16.7 ng/min/kg, the FHR returned to baseline by the end of the infusion (Fig. 5). Heart rate then increased by 31 ± 12 beats/min during the recovery period (p < 0.0001, Fig. 5). At the highest infusion rate, the FHR initially decreased by 14 ± 4 beats/min after the onset of infusion. Heart rate subsequently increased gradually over the infusion period. During the recovery period, heart rate reached its maximum of 58 ± 19 beats/min above control (p < 0.0005, Fig. 5). Thereafter, FHR gradually fell toward baseline.

Blood volume decreased significantly during the highest two infusion rates. The maximal decrease was $7.8 \pm 2.2\%$ after 25 minutes of angiotensin II infusion at a rate of 230.0 \pm 30.9 ng/min/kg (p < 0.0005). Linear regression analysis showed a strong relationship between the infusion rate and the change in blood volume at the end of the infusion (r = -0.83,

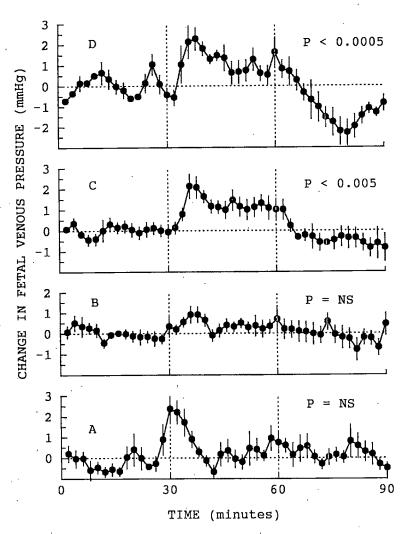


Fig. 3. Changes (mean ± SE) in fetal venous pressure during infusion of angiotensin II from 30 to 60 minutes. Infusion rates were 4.8 ± 1.1 (A, n = 6), 27.7 ± 4.6 (B, n = 7), 101.2 ± 16.7 (C, n = 6), and $239.0 \pm 30.9 \text{ ng/min/kg}$ (D, n = 4).

p < 0.00001, Fig. 6). In addition, a significant correlation existed between the change in fetal blood volume and arterial pressure responses during the last 20 minutes of the infusion (r = -0.86, p < 0.0001, Fig. 7). Similarly, a significant correlation was noted between the change in blood volume and the change in venous pressure at this time (r = -0.49, p < 0.05). However, with multivariate analysis the changes in fetal blood volume were statistically related to arterial pressure (p < 0.00001) but not to venous pressure. Blood volume remained reduced during the recovery period.

Plasma protein concentration increased significantly during the highest angiotensin II infusion rate, with the peak change being 0.27 ± 0.03 gm/dl (p < 0.05) after 25 minutes of infusion. There was no significant change in plasma protein concentration during the lowest or intermediate infusion rates.

Changes in fetal Pco2 and Po2 during or after an-

giotensin II infusion were nonsignificant, except for an increase in arterial Po₂ of 3.3 ± 0.9 mm Hg (p < 0.01) during the recovery period at the highest infusion rate. Fetal arterial pH decreased significantly during the recovery period after 27.7 ± 4.6 and 102.2 ± 16.7 ng/min/kg (p < 0.01). At 239.0 ± 30.9 ng/min/kg, the decrease began during the infusion and persisted into the recovery period (p < 0.001). The maximal observed decrease was 0.02 ± 0.01 pH units.

Plasma osmolality, Na+, Cl-, and K+ did not show significant changes during any infusion rate.

Comment

The purpose of this study was to explore dose-response relationships for the effects of angiotensin II on fetal arterial pressure, venous pressure, heart rate, and blood volume. To do this, we infused angiotensin II at mean rates of 4.8 \pm 1.1, 27.7 \pm 4.6, 101.2 \pm

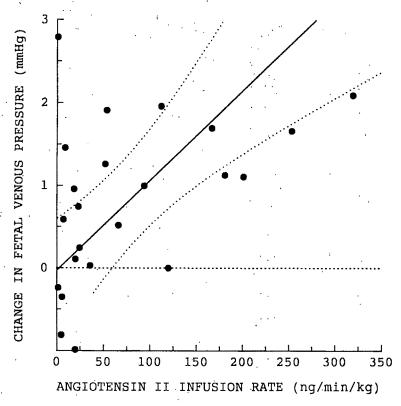


Fig. 4. Linear regression plot of change in fetal venous pressure averaged over last 20 minutes of angiotensin II infusion for each experiment (24 total) versus infusion rate of angiotensin II (r = 0.47, p < 0.05). Dashed lines, 95% Confidence interval about mean.

16.7, and 239.0 \pm 30.9 ng/min/kg of fetal weight for 30 minutes. Although fetal plasma concentrations were not available from this study, the plasma concentrations achieved during the infusions can be estimated from the work of other investigators. Specifically, Robillard et al.8 reported a fetal plasma clearance of infused angiotensin II of 170 ml/min/kg. From this it can be calculated that each nanogram per minute per kilogram infused would increase fetal plasma angiotensin II concentration by 5.9 pg/ml. Thus our four infusion rates would increase plasma concentration by approximately 28, 163, 603, and 1410 pg/ml, respectively. As most previous studies reported a basal fetal plasma concentration of 40 to 80 pg/ml with increments of several hundred picograms per milliliter under stress conditions.3.19 our infusions cover the physiologic range of fetal plasma angiotensin II concentrations.

The increase in arterial pressure that occurred during angiotensin II infusion appears to be a prompt and direct effect of angiotensin II, which, at the highest infusion rate, caused a 42% increase above control. Arterial pressure appeared to reach a steady state that persisted throughout the infusion period. With termination of the infusion, fetal arterial pressure rapidly returned to baseline values. Therefore, the arterial pressure response to angiotensin II appeared to be sus-

tained as long as circulating levels were elevated, but was teminated immediately as angiotensin II was cleared. This rapidity of onset of activity and subsequent clearance is consistent with the suggestion that angiotensin II is important in the initial response of the fetus to hypotension.^{1, 3, 19, 20}

In our experiments a maximal fetal arterial pressure response of approximately 21 mm Hg occurred at an infusion rate of 325 ng/min/kg (fig. 2). Other investigators infusing angiotensin II as a bolus21 or as a short (5 to 8 minutes) infusion9-11, 22 observed a similar increase in fetal arterial pressure at infusion rates comparable with those used in the current study. In addition, they also observed greater increases at higher infusion rates. Collectively, these studies suggest that the pressor effect of angiotensin II is dose dependent and increases linearly with increasing doses of angiotensin II. Thus findings with respect to fetal arterial pressure in the present experiment are consistent with the work of previous investigators. In addition, the responses are extended over a broader dose-response range for infusions lasting >5 minutes.

The observation of changes in central venous pressure of the fetus during angiotensin II infusion provides new information. The measured increases were subtle but significant. At the highest infusion rate, a

Fig. 5. Changes (mean \pm SE) in FHR during infusion of angiotensin II from 30 to 60 minutes. Infusion rates were 4.8 \pm 1.1 (A, n=7), 27.7 \pm 4.6 (B, n=7), 101.2 \pm 16.7 (C, n=6), and 239.0 \pm 30.9 ng/min/kg (D, n=4).

 1.5 ± 0.3 mm Hg increase in venous pressure was observed. This increase may indicate that the elevated arterial pressures were transmitted across the capillary bed or might indicate a direct effect of angiotensin II on venous resistance or capacitance vessels. This, coupled with the observation that the largest decrease in blood volume occurred simultaneously with the greatest increase in venous pressure, suggests that a venous constriction had occurred. The drop in venous pres-

sure during the recovery period after the highest infusion rate also appears to imply a decrease in vasoconstriction with termination of the angiotensin II infusion, because intravascular volume was decreased at that time.

The observation that fetal blood volume decreased in response to angiotensin II has not been previously reported. Blood volume most likely decreased as a result of increased capillary pressure and a shift of in-

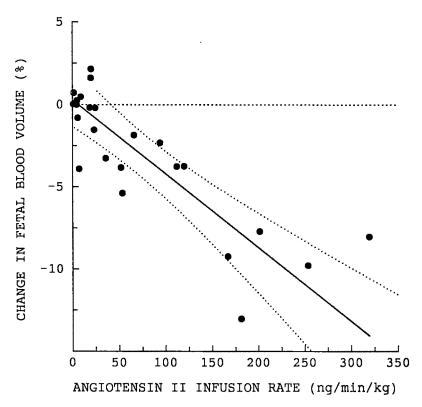


Fig. 6. Linear regression plot of change in fetal blood volume after 25 minutes of angiotensin II infusion versus infusion rate (r = -0.83, p < 0.0001). Dashed lines, 95% Confidence interval about mean.

travascular fluid into the interstitium. Fetal capillaries have a high permeability, and a significant increase in hydrostatic pressure would drive fluid across the capillary membrane.23 This is also consistent with the observed increases in plasma protein concentration. Blood volume remained reduced during the recovery period. This shows that return of the displaced fluid to the intravascular space was a gradual process.

In our study the FHR response to angiotensin II was biphasic in that there was an initial drop in heart rate, as much as 14 ± 4 beats/min at the highest infusion rate, followed by an increase within a few minutes. The initial decrease was probably baroreceptor mediated. The rise appears to be a direct effect of angiotensin II on the fetal heart, overriding the baroreceptor reflex. Because of the relative vascular hypertension, tachycardia cannot be attributed to the observed decrease in intravascular volume. In fact, both Iwamoto and Rudolph³ and Robillard et al. 19 showed in ovine fetuses that a decrease in fetal blood volume of as much as 20% did not produce a significant increase in FHR. In the current experiment, the persistent elevation in FHR observed after angiotensin II infusion may be secondary to the enhanced release or reduced reuptake of another cardiac chronotropic hormone, such as norepinephrine. This was suggested by our previous work¹⁴ in which the infusion of exogenous norepinephrine produced changes in FHR that were nearly identical to those observed in our study.

The observed changes in pH were slight, with the maximal change being only 0.02 pH units. Iwamoto and Rudolph⁷ and Robillard et al.⁸ reported no change in fetal pH, Pco2, or Po2 after angiotensin II infusion as high as 280 ng/min/kg. However, Yoshimura et al.¹⁰ reported a decrease in pH of 0.13 pH units and Po2 of 3.9 mm Hg with an increase in Pco₂ of 8.9 mm Hg after 5 to 7 minutes of angiotensin II at 5.73 µg/min. The effects of angiotensin II on umbilical vascular resistance and blood flow have been well documented. 9, 10, 22, 24 Angiotensin II increases umbilical vascular resistance and decreases flow. This alteration in umbilicoplacental flow may account for the slight changes in fetal pH observed in this study.

Our study suggests that differences in experimental design may be the cause for the differences in the FHR responses to angiotensin II reported by various investigators. Ismay et al.6 and Scroop et al.21 administered angiotensin II as a bolus to produce acute hypertension. The immediate baroreceptor response most likely produced a decrease in FHR. Such a finding was also seen by Clark et al.9 and Yoshimura et al.10,11 after a 5- to 7minute infusion of angiotensin II. However, bradycardia was only a transient response. Iwamoto and Rudolph⁷ and Robillard et al., with infusion rates sim-

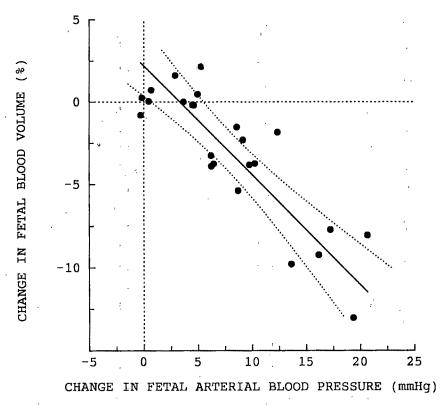


Fig. 7. Linear regression plot of change in fetal blood volume after 25 minutes of angiotensin II infusion versus change in fetal arterial blood pressure (r = -0.86, p < 0.0001). Dashed lines, 95% Confidence interval about mean.

ilar to the present study, reported an increase in FHR when a steady-state angiotensin II response was achieved. The short-term chronotropic cardiac response to angiotensin II appears to be an acute decrease in FHR mediated by the baroreceptor reflex, followed by an increase in FHR, possibly due to direct effects of angiotensin II on the fetal heart or increased release of endogenous cardiac catecholamines.

The response of the fetal cardiovascular system to angiotensin II bears similarities to that of norepinephrine. Norepinephrine infusion according to the same protocol as used in this study increased fetal arterial pressure in a dose-dependent manner.14 The venous pressure response to norepinephrine infusion was similar to the venous pressure response after angiotensin II. Like the angiotensin II infusion, the venous pressure effects were seen only at the higher norepinephrine infusion rates. The FHR responses to norepinephrine were nearly identical to those with angiotensin II, and a similar decrease in blood volume was observed. The similarities in the fetal cardiovascular responses between angiotensin II and norepinephrine may suggest that some of the cardiac effects of angiotensin II may be mediated by norepinephrine. Further investigation of any altered effect of angiotensin II in

the autonomically blocked fetus may support this possibility.

In summary, this study extends previous observations on the fetal arterial pressure responses to angiotensin II and provides new information about its effects on fetal venous pressure, heart rate, and blood volume. Collectively, these observations suggest that angiotensin II may be an important regulatory hormone for the entire fetal cardiovascular system.

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In vivo and in vitro effects of magnesium sulfate in the cerebrovascular bed of the goat

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The effects of magnesium sulfate in the cerebrovascular bed were studied both in vivo, by measuring cerebral blood flow in conscious nonpregnant goats, and in vitro, by recording isometric tension in isolated goat middle cerebral arteries. Injections of increasing doses (10 to 300 mg) of magnesium sulfate directly into the cerebral circulation elicited transient and dose-dependent increases in cerebral blood flow and decreases in cerebral vascular resistance. Similar results were obtained when increasing doses (0.3 to 3 gm/15 min) of magnesium sulfate were infused intravenously, although the vasodilatations reached a stable plateau that remained when the infusions finished. Cumulative addition of magnesium sulfate (10^{-5} to 3×10^{-2} mol/L) did not change the isometric tension of isolated arterial segments at resting tone, but relaxed in a concentration-dependent manner the arterial segments preconstricted with 10^{-6} mol/L prostaglandin F_{2n} . These results demonstrate that magnesium sulfate acts as a dilator in the cerebral circulation by acting directly on the cerebral arteries. This could explain, at least in part, its beneficial effects on preeclampsia-eclampsia. (AM J OBSTET GYNECOL 1991;165:1534-8.)

Key words: Magnesium sulfate, cerebral blood flow, cerebral arteries, goat

There is considerable evidence that cerebral vasospasm is involved in the pathogenesis of eclampsia. In fact, the earlier suggestion that the cerebral pathologic findings observed in eclampsia result from periods of vasoconstriction that induce focal necrosis and hemorrhages¹ has now been confirmed by cerebral angiographic and computed tomographic studies.²

In spite of the fact that magnesium sulfate is widely used to prevent and control eclamptic convulsions,^{3, 4} there is considerable controversy regarding its mechanism of action.⁵ Of particular interest is the hypothesis that magnesium could act in a similar way to organic Ca²⁺ entry blockers and thereby relieve cerebral vasospasm and prevent cell damage and cell death.⁶

At present little is known about the effects of magnesium sulfate on the cerebral circulation, because previous reports concerning this subject are limited to in vitro studies. The present work was undertaken to analyze the cerebrovascular effects of magnesium sulfate. Two experimental approaches were used, an in vivo preparation that permits continuous monitoring of cerebral blood flow in the conscious goat and an in vitro

vitro ^b Hos-Depar-

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Reprint requests: Dr. E. Alborch, Centro de Investigación, Hospital "La Fe," Avda. Campanar 21, E-46009-Valencia, Spain. 6/1/30950 preparation to record isometric tension in the isolated goat cerebral arteries.

Material and methods

Experimental model in vivo: Measurement of cerebral blood flow. Experiments were performed by following the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society (1980) and subscribed to by the Scientific Committee of the Centro de Investigación, Hospital "La Fe."

Seven nonpregnant female goats weighing 26 to 44 kg were used in this study. The goat was selected because its unique arterial supply to the brain permits measurement of the entire hemispheric blood flow. The operative procedure for measuring cerebral blood flow has been described previously.8 Briefly, the extracerebral blood flow conducted through the internal maxillary artery is eliminated by ligature and thrombosis of the extracerebral vessels originating in this artery (dental, ethmoidal, ophthalmic, and buccinator arteries). In these conditions virtually all the blood flow from the internal maxillary artery is destined for the brain through the ramus anastomoticus.9 Therefore an electromagnetic flow probe around the internal maxillary artery accurately measures the blood flow to the ipsilateral hemisphere. A catheter was introduced into the temporal artery and permanently fixed to permit injection or infusion of drugs into the internal maxillary artery. A snare-type occluder was placed on the external carotid artery, close to the temporal artery, to obtain zero-flow baselines. The external connecting leads from the flow probe, temporal artery catheter, and occluder were led out subcutaneously and secured to the goat's horn. Catheters were inserted in both the femoral artery and vein to measure the arterial blood pressure and allow blood sampling or infusion of drugs.

Experiments were performed in the conscious animal and started 3 to 5 days after surgery, at which time the goats had fully recovered and were in a steady cardiorespiratory state. The various measurements were taken with the goat in a large cage without restraints, except for a Lucite stock fitting loosely around the neck, which limited forward and backward motion. Once placed in the cage, the animal stood quietly during the experiments and showed no signs of any disturbance. Cerebral blood flow (pulsatile and mean), arterial blood pressure, and heart rate were continuously registered with a Hewlett Packard recorder (model 775&A). Cerebral vascular resistance was calculated as the mean arterial blood pressure in millimeters of mercury divided by cerebral blood flow in milliliters per minute × 100 gm tissue. After termination of the experiments the animals were killed by intravenous injection of 30 mEq potassium chloride, and the whole brain was removed from the skull and weighed for accurate calculation of cerebral blood flow in milliliters per minute × 100 gm of tissue.

The effects of magnesium sulfate (MgSO₄ · 7H₂O) were evaluated during both local intraarterial injections and systemic intravenous infusions. In the first set of experiments, increasing doses (10, 30, 100, and 300 mg) were injected into the internal maxillary artery. The doses were injected through the temporal catheter in a volume of 1 ml saline solution, and the catheter was immediately washed out with an additional 1 ml of the same saline solution. Control injections of saline solution never produced any artifact or subsequent change in cerebral blood flow. In the second set of experiments, increasing doses (0.3, 1, and 3 gm) were infused through the femoral vein catheter by means of a perfusion pump (Hoeschst PP50, rate 1 ml/min) over a period of 15 minutes each.

Experimental model in vitro: Recording of isometric tension. Three additional female goats, previously anesthetized with intravenous 2% thiopental sodium, were killed by intravenous injection of 30 mEq potassium chloride. The whole brain was rapidly removed and the middle cerebral arteries were dissected free and cut into cylindric 4 mm segments. Each segment was set up in a 5 ml organ bath, and two L-shaped stainless steel pins were introduced through the arterial lumen. One pin was fixed to the organ bath wall, and the other was connected to a strain gauge for isometric tension recording. The vascular preparations were

maintained at 37° C in a Ringer-Locke solution gassed with 95% oxygen and 5% carbon dioxide to give a pH of 7.3 to 7.4. The composition of the nutritive medium was as follows: sodium chloride, 120 mmol/L; potassium chloride 5.4 mmol/L; calcium chloride, 2.2 mmol/L; magnesium chloride, 1.0 mmol/L; sodium bicarbonate, 25 mmol/L; and glucose, 5.6 mmol/L. A resting tone of 1 gm was applied, and the arterial segments were allowed to equilibrate during a period of 60 to 90 minutes. Tension was readjusted when necessary and the bath fluid was changed every 15 minutes.

Responses to magnesium sulfate (10^{-5} to 3×10^{-2} mol/L) were obtained in a cumulative manner from arterial segments at resting tone and from arterial segments that had been previously contracted with a single dose of 10^{-5} mol/L prostaglandin $F_{2\alpha}$ (Sigma). The concentration-response curve for magnesium sulfate started once the effect of prostaglandin F2a reached a steady state (active tone).

Analysis of the results. The cerebral blood flow or cerebral vascular resistance values were expressed as a percentage of their respective resting values. For the experiments on intraarterial administration, seven dose-response curves were obtained on seven animals. For the experiments on intravenous infusion, each dose was infused over a period of 15 minutes, and the various parameters were measured at 5-minute intervals (three during infusion plus two additional ones once the pump was stopped). This experiment was repeated seven times on seven goats. Statistical analysis was performed by using the analysis of variance, followed by the least-significant differences test, when appropriate. A probability value of <5% was considered to be significant.

The tension values were expressed either in absolute terms (milligrams) or as a percentage of active tone. Six concentration-response curves in nonpreconstricted arterial segments, and 18 concentration-response curves in preconstricted arterial segments were obtained. For each concentration-response curve, the concentration of magnesium sulfate that produced one half the effect reached with the highest dose ("apparent" EC50) was calculated, since no maximum effect could be reached. The mean EC₅₀ and its confidence limits (95% interval) were calculated by obtaining the mean and confidence limits of the negative logarithms of EC50 values (pD2), because they fit tightly to a normal distribution.

Results

In vivo. Injections of increasing doses of magnesium sulfate (10, 30, 100, and 300 mg) directly into the cerebral circulation of the unanesthetized goat produced immediate, transient, and dose-dependent increases in cerebral blood flow from the dose of 100 mg. No significant changes in arterial blood pressure and heart

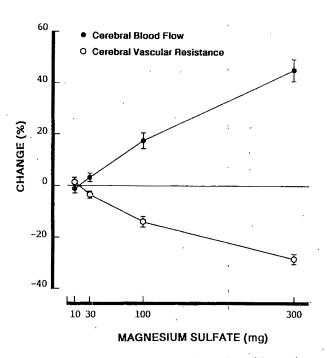


Fig. 1. Effects of intraarterial administration of increasing doses of magnesium sulfate (n = 7) on cerebral blood flow and cerebral vascular resistance in conscious nonpregnant goat. Changes in both parameters are expressed as percentage of their corresponding resting values. Data points represent means \pm SEM.

rate were recorded during the cerebral blood flow increases; therefore dose-dependent reductions in cerebral vascular resistance were obtained. Fig. 1 shows the dose-response curve for magnesium sulfate on both cerebral blood flow and cerebral vascular resistance. Table I shows the effects of 100 mg magnesium sulfate on cerebral blood flow, arterial blood pressure, heart rate, and cerebral vascular resistance.

Intravenous infusions of magnesium sulfate (0.3, 1, and 3 gm/15 min) elicited increases in cerebral blood flow in the unanesthetized goat. Because no significant changes in arterial blood pressure and heart rate were recorded along with the increased cerebral blood flow, reductions in cerebral vascular resistance were obtained. These vasodilatory effects are shown in Fig. 2. Whereas those of 0.3 and 1 gm reached a significant, stable plateau 10 minutes after start of the infusion, that of 3 gm reached it at 5 minutes. These changes remained for 10 minutes after the infusions were stopped. The magnesium plasma levels were determined in one animal at resting state (control) and after completion of the three infusions. The concentrations were 1.7 mg/dl (control), 2.07 mg/dl (0.3 gm), 2..81 mg/dl (1 gm), and 6.67 mg/dl (3 gm).

In vitro. At resting tone, cumulative addition of magnesium sulfate (10^{-5} to 3×10^{-2} mol/L) did not produce sizeable changes in vascular tension. Prostaglan-

din $F_{2\alpha}$ (10⁻⁵ mol/L) produced a sustained contraction (active tone) of 2036 \pm 997 mg (mean \pm SD). In these preconstricted arteries, magnesium sulfate elicited well-defined relaxations that were concentration dependent in magnitude (Fig. 3). The mean EC₅₀ was 5.7 (3.8 to 8.7) \times 10⁻³ mol/L, and the mean maximum effect was $-82.4 \pm 3.6\%$ (percentage of active tone).

Comment

It has long been recognized that vascular spasm may be an important component in the pathogenesis of preeclampsia-eclampsia. Actually, vasospasm has been directly observed in the nail beds, bulbar conjunctivae, and retinal vessels of eclamptic females¹⁰ and signs of coronary artery spasm also have been observed in patients who die of eclampsia. Recent angiographic and computed tomographic studies demonstrate that vascular spasm also affects the brain.²

In the light of this evidence, one may ask about the mechanism of action by which magnesium sulfate prevents and controls eclamptic convulsions. It is tempting to hypothesize that the way in which magnesium relieves cerebral vasospasm is similar to the way organic calcium entry blockers do it. Magnesium has been shown to counter the actions of calcium in many situations, which makes it nature's physiologic calcium blocker¹²; on the other hand, there is now considerable evidence obtained both in vivo13 and in vitro14 that calcium entry blockers inhibit cerebral vasospasm. With this argument, one might expect magnesium to act as a cerebral vasodilator because calcium entry blockers increase cerebral blood flow15 and relax isolated cerebral arteries.14 That hypothesis is the one that we tested in this work.

The results reported here demonstrate unequivocally that, regardless of the route of administration (directly into the cerebroarterial supply or systemically), magnesium sulfate increased cerebral blood flow in the unanesthetized goat with no concomitant changes in arterial blood pressure and heart rate. Our observations on arterial blood pressure and heart rate agree with those reported previously in humans16, 17 and sheep¹⁸ and demonstrate that the vasodilatory action of magnesium sulfate results from a decrease in cerebral vascular resistance. These results resemble those reported previously in pregnant monkeys,17 which show that magnesium sulfate increases uterine blood flow without altering perfusion pressure and cardiac output, which indicates a decrease in uterine vascular resistance. In contrast, blood flow to other organs such as kidney was not changed by magnesium sulfate. Taken together, all of this evidence suggests that magnesium sulfate elicits a redistribution of cardiac output by favoring the irrigation of some organs.

The question arises as to the mechanism by which

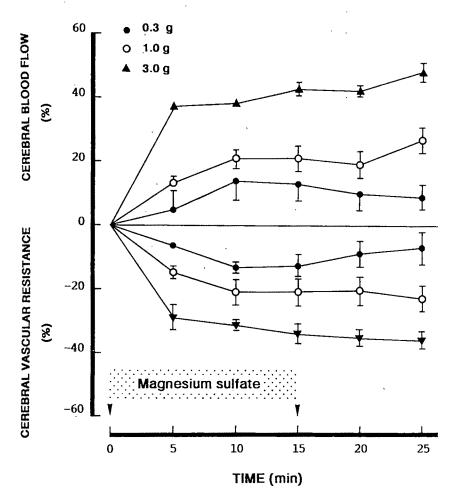


Fig. 2. Effects of intravenous infusion of increasing doses of magnesium sulfate (n = 7) on cerebral blood flow and cerebral vascular resistance in conscious nonpregnant goat. Changes in both parameters are expressed as percentage of their corresponding resting values. Data points represent means ± SEM.

Table I. Effects of magnesium sulfate on cerebral blood flow, arterial blood pressure, heart rate, and cerebral vascular resistance when it is injected into the internal maxillary artery of the unanesthetized goat

	Control	Magnesium sulfate (100 mg)
Cerebral blood flow (ml/min × 100 gm)	· 112.7 ± 14.8	132.4 ± 11.1*
Arterial blood pressure (mm Hg)	118.7 ± 10.8	119.1 ± 6.4
Heart rate (beats/min)	82 ± 9	85 ± 7
Cerebral vascular resistance (mm Hg × 100 gm × min/ml)	1.06 ± 0.11	$0.91 \pm 0.06*$

Values are means ± SD obtained from seven goats.

magnesium sulfate increases cerebral blood flow. A blood-brain barrier for magnesium seems to exist, since cerebrospinal fluid levels of magnesium do not change after administration of magnesium sulfate.19 Therefore it should be accepted that, even if only small amounts of magnesium sulfate diffuse and reach the smooth muscle cells, it acts mainly at the endothelial level. Actually, magnesium sulfate stimulates in vitro the production of prostacyclin (a potent vasodilator) by endothelium of human umbilical vein.20 Because prostacyclin is also produced by and released from the endothelial cells in the brain,21 it seems reasonable to hypothesize that magnesium sulfate acts, at least in part, through that mechanism.

^{*}Significantly different from control values, p < 0.01.

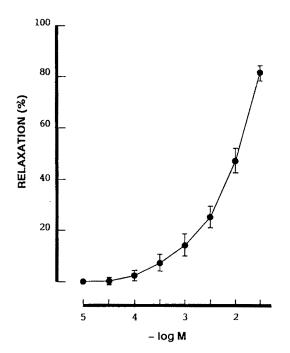


Fig. 3. Concentration-response curve for magnesium sulfate (n = 18) in isolated goat middle cerebral artery preconstricted with prostaglandin $F_{2\alpha}$ (10⁻⁵ mol/L). Relaxation values are expressed as percentage of active tone. Data points represent means \pm SEM.

Our results obtained in vitro show that magnesium sulfate relaxes the isolated cerebral arteries in a concentration-dependent manner. Magnesium has been shown to relax a number of arterial preparations,²² the only exception being femoral arteries, which contract when magnesium sulfate reaches the highest concentrations.⁷ With regard to the cerebral arteries, our results are in close agreement with those obtained in a previous work with feline cerebral arteries,⁷ both in the EC₅₀ and the maximum effect values. The importance of this finding resides in the fact that it confirms the conclusion reached in vivo that magnesium sulfate acts directly on the cerebral arteries and causes them to relax.

In summary, we present experimental evidence obtained both in vivo and in vitro that shows that magnesium sulfate is a dilator of the cerebral circulation. This supplies a rationale for its beneficial effects on preeclampsia-eclampsia, although its ability to relieve cerebral vasospasm should be assessed.

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The ability of recombinant tissue plasminogen activator to inhibit post-radical pelvic surgery adhesions in the dog model

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We investigated the ability of recombinant tissue plasminogen activator to inhibit post–radical pelvic surgery adhesions formation in 40 adult female canines undergoing radical hysterectomy, bilateral salpingo-oophorectomy, omentectomy, resection of pelvic and abdominal peritoneum, and placement of a peritoneal access catheter. Immediately after operation one half of animals received either recombinant tissue plasminogen activator, 1 mg/kg weight, diluted in 9 ml sterile normal saline solution per milligram of the plasminogen activator or 10 ml vehicle per kilogram intraperitoneally every 12 hours for a total of 10 doses. A single control animal died postoperatively of complications of intestinal obstruction. No bleeding abnormalities were noted in either group of animals. Four weeks after surgery, animals underwent reexploration and adhesions were quantified. Adhesion scores for the animals treated with recombinant tissue plasminogen activator (n = 20; mean score, 1.29 \pm 1.97; median, 0.6) were significantly less than for control animals (n = 19; mean score, 4.64 \pm 3.71; median, 3.86; p = 0.03). Whereas recombinant tissue plasminogen activator appears to effectively prevent post–radical pelvic surgery adhesions in this canine model, phase I and II trials in humans will be required to determine safety and clinical benefit. (Am J Obstet Gynecol 1991;165:1539-42.)

Key words: Post-radical pelvic surgery adhesions, recombinant tissue plasminogen activator

Radical pelvic surgery, when used in the treatment of patients with gynecologic malignancies, has many well-defined side effects. These include, but are not limited to, the following: intestinal and ureteral obstruction, bowel dysfunction, fistula formation, and chronic abdominal-pelvic pain. The development of postsurgical adhesions also interferes with the intraperitoneal distribution of fluids. This interference limits the use of many intraperitoneal treatment modalities.^{1, 2}

Many potential adhesion-preventing agents have been investigated throughout the history of surgery.³ None, including the more recently advocated barrier methods, have demonstrated effectiveness at limiting adhesion formation after a radical pelvic resection similar to that performed in patients treated for advanced ovarian malignancies.^{4, 5} Human recombinant tissue plasminogen activator is a thrombolytic agent that binds to fibrin and activates plasminogen to plasmin.⁶ This plasmin is the primary enzyme responsible for the pro-

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Reprint requests: F.J. Montz, MD, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, UCLA Center for Health Sciences, 10833 Le Conte, Los Angeles, CA 90024-1740. 6/1/31297 teolytic degradation of fibrin. The persistence of excessive fibrin has been proposed as a pathophysiologic basis of adhesion formation. Recent investigations have suggested that recombinant tissue plasminogen activator, when applied topically, may be effective in limiting postoperative adhesion development. So as to confirm or refute these findings, we investigated the ability of recombinant tissue plasminogen activator to limit the formation of postoperative abdominal-pelvic adhesions when used in an animal model that underwent a radical resection of the pelvic viscera and peritoneum.

Material and methods

Forty adult female mongrel canines that had not previously undergone intraperitoneal surgery were used. Animals were housed at the University of California at Los Angeles vivarium. All animal procedures were performed in accordance with the standards described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, in compliance with the Federal Animal Welfare Act.

Mean animal weight was 21 kg (range, 18.5 to 23.5). Animals were quarantined for 10 days, during which time they were allowed access to water and chow ad libitum, then fasted for 24 hours before surgery. After induction of general endotracheal anesthesia with pentobarbital sodium, 10 mg/kg, and assisted ventilation with a volume ventilator and an Fio₂ of 40%, animals

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Table I. Adhesion grade

Grade 0 (0 points) Grade I (1 point)	No adhesions Avascular; easily lysed and fail-
Grade II (3 points)	ing to bleed Vascular; easily lysed but bleeds at time of lysis
Grade III (5 points)	Thick; requires extensive sharp surgical dissection

Modified from Shimanuki et al.13,

underwent exploratory celiotomy. The animals' anterior abdominal wall, right chest wall, and back to the level of the scapula were shaved and prepped with a standard povidone-iodine solution. With the animals in a left lateral decubitus position, with an aseptic technique, a 1 cm stab incision was made below the scapula on the right side. Through this incision an intraperitoneal catheter was tunneled under the skin of the back and chest wall, then brought through the skin immediately below the right costal margin. The animals were repositioned in a supine position, reprepped, and draped with sterile technique. The abdomen was sharply opened by a midline incision from the umbilicus to the symphysis pubis. Intraperitoneal exploration was performed, and a total radical abdominal hysterectomy, bilateral salpingo-oophorectomy, and infracolonic omentectomy were performed. The pelvic peritoneum was removed along with the peritoneum from the lower one third of the anterior abdominal wall. Surgery was performed with bipolar electrocautery, both for cutting and for coagulation of individual bleeding sites. Free vascular pedicles were ligated with 2-0 silk ties. The vaginal cuff was closed with interrupted 2-0 chromic sutures.

Before closing the abdomen, a subcutaneous tunnel was made and the intraperitoneal catheter brought from the subcostal site to the most inferior aspect of the incision. The catheter was then bluntly brought through the abdominal wall fascia and placed into the peritoneal cavity. Adequate catheter length was left intraperitoneally to allow the tip to lie in the cul-de-sac. After assuring hemostasis and a correct sponge and needle count, the anterior abdominal wall was closed with I-0 Maxon (Davis and Geck, Inc., Manati, Puerto Rico) sutures in a running Smead-Jones technique. The skin was closed with subcuticular 3-0 Dexon (Davis and Geck) sutures. The stab wounds on the chest and abdominal wall were closed with 3-0 Dexon sutures in an interrupted manner. The catheter was secured at the site of entrance into the subcutaneous tunnel with 3-0 Prolene (Ethicon, Inc., Somerville, N.J.).

In a randomized manner, after the completion of surgery, the animals were divided into two groups. One half of the animals received a dose of 1 mg/kg of recombinant tissue plasminogen activator (Activase, Al-

teplase, 1 mg/ml supplied by Genentech Inc., South San Francisco), which was diluted with 9 ml of sterile normal saline solution for each milligram of recombinant tissue plasminogen activator, intraperitoneally every 12 hours for a total of 10 doses. This dose and dosing schedule have been shown to have a maximal adhesion prevention effect in a rabbit uterine horn model.11 The initial intraperitoneal bolus was given immediately after completion of surgery. Control animals were similarly treated with 10 ml of normal saline solution (inert vehicle) per kilogram intraperitoneally every 12 hours for a total of 10 treatments. After the tenth instillation of either recombinant tissue plasminogen activator solution or vehicle alone, the catheters were removed and the exit site sutured closed with 3-0 Dexon. The length of treatment, 5 days, was selected in light of the fact that organization of fibrinous adhesions does not take place until that time12 and that by increasing the duration of treatment to 5 days a maximum adhesion prevention effect can be obtained.11

The animals were observed in the postoperative recovery area for 24 hours after surgery and recovery from general anesthesia. During that period, they were allowed free access to water, but food was withheld. After the immediate postoperative recovery, the animals were returned to the general dog run and allowed access to food and water as desired. All animals were observed on a twice-daily basis. Abdominal incisions and catheter sites were evaluated for signs of bleeding, infection, or breakdown. The animals also were observed for signs of abnormal bleeding, gastrointestinal or urinary dysfunction, and general recovery and activity levels.

Four weeks after the initial surgery, anesthesia followed by euthanasia was effected with intravenous pentobarbital sodium (100 mg/kg). While under anesthesia, but before being killed, the animals underwent exploration through a supraumbilical incision. The incision was extended caudally and adhesions were quantified and scored for the anterior abdominal wall incision and pelvis. Adhesion scoring was performed by a surgeon who was blinded to the treatment (recombinant tissue plasminogen activator or vehicle) that the animal received. The pelvis was divided into four quadrants with the vaginal apex dividing the pelvis into anterior-posterior and lateral halves. Adhesions were recorded as the percentage of surface area covered in each of the five regions (abdominal wall incision and four pelvic quadrants). Adhesions were scored according to type (Table I). Abnormal findings (abscess formation, lymphadenopathy, foreign body reaction, etc.) and the integrity of the wound were similarly recorded.

With a modification of a previously reported method, 18 adhesion scores for each of the five locales

Table II. Adhesion scores

	Grade	I	Grade	II	Grade .	III	Total	!
	Mean	Median	Mean	Median	Mean	Median	Mean	Median
Recombinant tissue plasminogen activator— treated animals (n = 20)	0.29 ± 0.25	0.24	0.19 ± 0.38	. 0	0.81 ± 1.20	0.31	1.29 ± 1.97	0.60
Vehicle-treated animals (n = 19)	0.63 ± 0.28	0.64	0.75 ± 0.77	0.61	3.17·± 2.69	2.82	4.64 ± 3.71	3:86
p Value	0.017	7	0.048	3	0.01		0.03	

were calculated. This was done by multiplying the assigned points for each adhesion grade by the percentage of the surface area involved with that specific grade of adhesion. A value of 1.00 was used to equal 100% involvement. By summing the data from all five locales, a grade-specific adhesion score and a total adhesion score were obtained. Statistical analysis was performed on log transformation of the adhesion scores with the Student t or Wilcoxon nonparametric test, where indicated.

Results

There were no intraoperative deaths in this series. One control animal did die on the eighth postoperative day; necropsy demonstrated extensive intraperitoneal adhesions, a small bowel obstruction, and a necrotic, perforated segment of ileum. No bleeding abnormalities were noted in either the experimental or control animals. However, serum recombinant tissue plasminogen activator levels and coagulation studies were not performed. No abscesses, fistulas, or fascial defects or hernias occurred in either group.

Adhesion scores for the recombinant tissue plasminogen activator-treated animals (n = 20; mean adhesion score, 1.29 ± 1.97 ; median, 0.6) were significantly less than for control animals (n = 19; mean adhesion score, 4.64 ± 3.71 ; median, 3.86; p = 0.03). This finding was consistent for all types and locales of adhesions (Table II).

Comment

Postsurgical remesothelialization of the peritoneal cavity is the result of a complex series of reactions.14 Peritoneal injuries associated with ischemia, hemorrhage, foreign body, or infection are known to disrupt the equilibrium between the normal fibrin deposition and fibrinolysis, thus increasing adhesion development.15-18 It is this latter phenomenon that has stimulated investigators to perform studies to determine if locally administered recombinant tissue plasminogen activator could be successful at curtailing postsurgical adhesion formation.8-10

The ability of recombinant tissue plasminogen acti-

vator to limit adhesion formation is probably due to its success at reestablishing the normal equilibrium of fibrin deposition and fibrinolysis by increasing the local level of plasminogen activator activity. Multiple investigators have proposed that this effect is the most important link in a final common pathway by which many antiadhesion agents are able to decrease postsurgical adhesion formation.19.20

High doses of recombinant tissue plasminogen activator administered as a thrombolytic agent have been shown to cause decreases in systemic fibrinogen and increases in fibrin degradation products.21 Possible complications from the intraperitoneal use of recombinant tissue plasminogen activator, such as excessive bleeding and the prevention of wound healing, were not observed in this study. However, future studies will be needed to accurately assess coagulation and hematologic parameters. The fact that no animals evidenced any apparent bleeding abnormality may be explained by the fact that there could be fairly rapid absorption of the recombinant tissue plasminogen activator into the systemic circulation with subsequent rapid clearance from the circulation or that degradation may occur in the peritoneal cavity. Similarly, because recombinant tissue plasminogen activator preferentially activates fibrin-bound plasminogen,22 the generation of free plasmin in the peritoneum would be minimized and the potential for any bleeding abnormality limited. Although no animals in either group were completely adhesion-free, a significant decrease was seen in all grades of adhesions in the recombinant tissue plasminogen activator-treatment group. Theoretically, optimization of dose and treatment duration may improve the reduction of adhesions with this therapy.

The discovery that recombinant tissue plasminogen activator has a demonstrated capacity to limit adhesion formation in this unique model has stimulated speculation that it may be effective when given intraperitoneally in women who have undergone radical oophorectomy and resection of pelvic and lower abdominal peritoneum as part of the treatment for metastatic ovarian malignancies. Should such an effect be demonstrated, it would open the way to increased use of adjuvant intraperitoneal treatment modalities immediately after surgery. It is during this period when tumor volume is the lowest and cell growth fraction the highest and thus may be the time when any residual tumor is most susceptible to cytotoxic therapy.

At present, no agent has been demonstrated to be effective in either the animal or human model at limiting adhesions after radical pelvic surgery. In light of this fact, adhesion prevention agents are not usually used by the radical pelvic surgeon, although their benefit beyond that of facilitating the use of intraperitoneal therapeutic agents is self-evident (decrease in postoperative bowel obstructions, ureteral obstructions, chronic pelvic pain syndromes, etc.).

In conclusion, it is important to remember that our results come from a limited animal trial. At present there are no available data regarding the safety of intraperitoneal recombinant tissue plasminogen activator in humans; phase I and phase II clinical studies will be needed to thoroughly evaluate the safety and risks of intraperitoneal recombinant tissue plasminogen activator in the prevention of post—radical pelvic surgery adhesion formation in human subjects.

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Effects of magnesium and terbutaline on contractility and K⁺ uptake in isolated human uterine muscle

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Mg⁺⁺ (3 and 6 mmol/L), the β_2 -adrenergic agonist terbutaline (1 and 10 μ mol/L), and dibutyryl cyclic adenosine 5'-monophosphate (0.1 and 1 mmol/L) suppressed spontaneous activity and the increase in contractile activity induced by ouabain or K⁺-free buffer in isolated human pregnant myometrium. The ouabain-suppressible rubidium 86 or potassium 42 uptake was unaffected by the presence of Mg⁺⁺ (3 and 6 mmol/L), the β_2 -adrenergic agonist terbutaline (1 and 10 μ mol/L), or dibutyryl cyclic adenosine 5'-monophosphate (1 mmol/L). However, loading of the strips with Na⁺ and incubation in high K⁺ induced a fivefold increase in rubidium 86 uptake. On the basis of these flux rates, our previous data on the total concentration of sodium-potassium pumps in the human myometrium, and an estimated maximum transport rate of the sodium-potassium pump of 8900 K⁺ ions per minute at 30° C, it could be calculated that the sodium-potassium pump in the Na⁺-loaded strips reached around 80% of its maximal rate. Taken together, these results showed that the relaxant effects of Mg⁺⁺, terbutaline, and dibutyryl cyclic adenosine 5'-monophosphate on human myometrium are not due to a stimulation of active sodium-potassium transport. (AM J OBSTET GYNECOL 1991;165:1543-51.)

Key words: Human myometrium, Mg⁺⁺, β₂-adrenergic receptor agonist, cyclic adenosine 5'-monophosphate, sodium, potassium, adenosine triphosphatase

The β₂-adrenergic receptor agonist terbutaline is widely used as a tocolytic agent for the treatment of premature labor contractions, but it is well known that it is associated with several side effects. Since the late 1950s, when it was recognized that magnesium sulfate possesses tocolytic effects, the use of this agent has increased in the treatment of premature contractions. Several studies have compared the effectiveness of terbutaline and magnesium sulfate as tocolytic agents. No major differences concerning their ability to arrest labor contractions were found, but magnesium sulfate seemed to produce fewer side effects.

The exact mechanisms of action of Mg⁺⁺ and terbutaline on the myometrium are not clear. Experimental studies on human myometrium⁷⁻¹⁰ have demonstrated that selective β_2 -adrenergic receptor agonists inhibit uterine contractions, and it is generally believed that these effects are mediated through an increase in the intracellular concentration of cyclic adenosine 5'-monophosphate (cAMP).¹¹ Scheid et al.^{12, 13} found that

active electrogenic sodium-potassium transport in toad stomach smooth muscle was stimulated by the β -adrenergic agonist isoproterenol and suggested that the ensuing decrease in intracellular Na+ favored Na+Ca++ exchange, leading to decreased intracellular Ca++ and relaxation. A similar mechanism of action for β_2 -adrenergic receptors has been suggested in other smooth muscle preparations. However, in rat uterine muscle there is no reported evidence that the relaxant effects of isoproterenol should be due to a stimulation of the sodium-potassium adenosine triphosphatase. $^{18-21}$

The mechanism of action of Mg^{++} on human myometrium is not yet clarified. In rat myometrium Mg^{++} was shown to potentiate the actions of β_2 -adrenergic receptor agonists, 22 and in vascular smooth muscle from animals $^{23,\ 24}$ and man $^{25,\ 26}$ and in human nonpregnant myometrium 27 Mg^{++} was shown to interfere with Ca^{++} entry through the cell membrane and with intracellular Ca^{++} mobilization. Optimum function of the sodium-potassium adenosine triphosphatase depends on the presence of Mg^{++} at low concentrations, but whether Mg^{++} in concentrations reached during therapy (2 to 4 mmol/L) may influence active sodium-potassium transport is not known.

In our study we have examined the possible effects of Mg⁺⁺, terbutaline, and dibutyryl cAMP on active sodium-potassium transport in human pregnant myometrial strips obtained at term. Furthermore, the effects of these agents on contractile activity caused by

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sodium-potassium adenosine triphosphatase inhibition by K⁺-free buffer or ouabain were studied.

Material and methods

The study was approved by the local ethical committee according to the Helsinki Declaration II. All patients gave informed consent. In 10 pregnant women a biopsy of the uterine muscle was performed in the upper part of the isthmic incision at elective term cesarean section performed because of breech presentation (n = 5), genital herpes (n = 1), or previous cesarean section (n = 4). In two instances a biopsy specimen was obtained from the fundus region as well. The specimens were immediately placed in ice-cold Krebs-Ringer solution and transported to the laboratory. Under a stereomicroscope, smooth muscle strips (diameter, 1 mm; length, 4 to 5 mm) were dissected along the fiber direction with microscissors. To ensure comparability the strips prepared from each biopsy specimen were used for measurement of isometric tension and ion fluxes.

Isometric tension. The contractile performance of the myometrial strips was assessed essentially as previously described.28 By means of silk sutures (6-0) at either end, the preparations were mounted horizontally between two small, L-shaped hooks in 5 ml thermostatregulated organ baths (37° C) containing Krebs-Ringer bicarbonate buffer continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide, resulting in a final pH of 7.4. One of the hooks was attached to a force transducer (Grass Ft 03) for measurement of isometric tension, and the other was connected to a sledge, which allowed adjustment of length of the strip. Tension was recorded by a six-channel Beckman R611 polygraph. A small preload (0.1 mN) was applied to the strips, and the distance between the two knots of the silk sutures was measured by a stereomicroscope equipped with an ocular micrometer and was defined as the resting length. Adjustment was then performed to secure a distance between the two knots of 160% of the resting length. In separate length-tension experiments this was found to be optimum for mechanical performance (unpublished data). After the equilibration period, contractions were repeatedly induced by K⁺ (126 mmol/L) until reproducible (<10% variation between two successive contractions), and then the experiments commenced.

Ion fluxes

Potassium 42 or rubidium 86 uptake: All experiments were carried out with myometrial strips weighing 15 to 20 mg. Incubations took place at 30° C under continuous gassing with a mixture of 95% oxygen and carbon dioxide in a volume of 2 to 3 ml Krebs-Ringer bicarbonate buffer (pH 7.4) containing ⁴²K (0.5 μCi/ml) or ⁸⁶Rb (1 μCi/ml). Most experiments were carried out

with 86Rb. This is a tracer for potassium, but because of its much longer half-time, it is more convenient than ⁴²K. Immediately after preparation, the strips were equilibrated in the standard incubation medium for at least 30 minutes. This was followed by preincubation for 15 minutes in the absence or presence of ouabain (I mmol/L). This concentration has earlier been shown to allow full saturation of all tritium-ouabain binding sites in the human myometrium.29 Thereafter the myometrial strips were incubated for periods lasting between 2 and 60 minutes without or with ouabain (1 mmol/L) present. Incubation was followed by washout four times for 15 minutes each at 0° C in Na+-free Tris-sucrose buffer (Tris hydrochloride, 10 mmol/L; sucrose, 263 mmol/L; pH 7.4) so as to remove extracellular Na+ and tracer. The strips were then blotted, weighed, homogenized in 2 ml of 0.3 mol/L trichloroacetic acid, and taken for counting of 42K or 86Rb activity by Cerenkov radiation.

In some experiments myometrial strips were loaded with Na+ according to a method earlier developed.30 Incubation three times for 30 minutes in buffer in which all Ca++, Mg++, and K+ had been replaced by an equimolar amount of Na+, and 0.5 mmol/L ethylene glycol-bis (β-amino ethyl ether) N,N,N',N'-tetraacetic acid added so as to cause further reduction in extracellular Ca++. This was followed by incubation for 30 minutes in the same buffer with the addition of sodium chloride (100 mmol/L), and a 15-minute wash in normotonic Ca++-, Mg++-, and K+-free buffer containing 0.5 mmol/L ethylene glycol-bis (β-amino ethyl ether) N,N,N',N'-tetraacetic acid with or without ouabain (1 mmol/L). As calculated from measurements of the total Na+ content and the carbon 14-sucrose space, this procedure resulted in intracellular Na+ and K+ concentrations of 136 and 27 mmol/L, respectively. Hereafter, the strips were incubated for 10 minutes in the absence or presence of ouabain (1 mmol/L) in buffer in which all Na+ was replaced by K+ (151.2 mmol/L). Finally, the strips were washed, weighed, homogenized, and counted as described above. The ouabain-suppressible 42K or 86Rb uptake was calculated as the difference between the values obtained in the absence and in the presence of ouabain (1 mmol/L).

⁴²K efflux. This was measured essentially as described earlier for rat soleus muscle.³¹ After equilibration, the strips were preloaded for 60 minutes at 30° C in standard buffer containing ⁴²K (2 μCi/ml). The washout of ⁴²K was followed by transferring the strips through a series of tubes without isotope. Thereafter the strips were blotted, weighed, homogenized in 0.3 mol/L trichloroacetic acid and taken for counting of ⁴²K activity. By adding successively the activity in the washout tubes to that in the strips by the end of the experiment, the ⁴²K activity in the strips at each transfer was calculated.

On the basis of these values the fractional loss of 42K was calculated for each washout period.

 Na^+-K^+ contents. Strips were homogenized in 2 ml trichloroacetic acid (0.3 mol/L) and centrifuged. The Na+-K+ contents of these extracts were determined with a Radiometer FLM3 flame photometer with lithium as internal standard.92

Statistics. For ion flux experiments, after homogeneity of variance was ascertained, significance of difference was assessed by one-way analysis of variance or by the two-tailed t test for groups of nonpaired observations with a level of significance of 0.05.

Solutions. Krebs-Ringer bicarbonate buffer contained the following: 120.2 mmol/L sodium chloride, 25.1 mmol/L sodium bicarbonate, 4.7 mmol/L potassium chloride, 1.2 mmol/L potassium orthophosphite, 1.2 mmol/L magnesium sulfate, 1.3 mmol/L calcium chloride, and 5 mmol/L D-glucose. In K+-rich Krebs-Ringer bicarbonate buffer for tension measurement (126 mmol/L K+), sodium chloride was replaced by an equimolar amount of potassium chloride. In K+-rich Krebs-Ringer bicarbonate buffer for ion flux studies (151.2 mmol/L K+), both sodium chloride and sodium bicarbonate were replaced by equimolar amounts of potassium chloride and potassium bicarbonate, respectively.

Drugs. Drugs used were terbutaline (Bricanyl, Draco), dibutyryl cAMP N6-21-0-dibutyryladenosine 3:'5:'-cyclic monophosphate (Sigma), ouabain (G-strophanthin, Merck), forskolin (Sigma), and insulin (Novo-Nordisk).

Results

Isometric tension. Only myometrial strips that exhibited spontaneous contractions (about 95% of the preparations) were used for these experiments. Regular spontaneous activity consisting of phasic contractions, sometimes with superimposed minor oscillations at the peak, developed within I hour of equilibration with a frequency of two to three contractions per 10 minutes (Fig. 1, a). The pattern of contractile activity showed interindividual variation, therefore spontaneous activity and the effects of all agents tested are shown for preparations obtained from six different donors. Depolarization of the myometrial strips with a K+rich Krebs-Ringer buffer (126 mmol/L K+) produced a contraction consisting of a rapidly developing peak followed by a second phase with a steady tension amounting to about 20% of the initial peak. The maximum amplitude of the spontaneous contractions was about 90% of the K+-induced peak amplitude. The amplitude of tension produced by the spontaneous contractile activity varied between 4 and 20 mN.

Fig. 1 shows the effects of Mg++, terbutaline, and dibutyryl cAMP on the spontaneous contractile activity. Mg++ (added to final concentrations of 3 and 6 mmol/L) invariably abolished the contractions, irre-

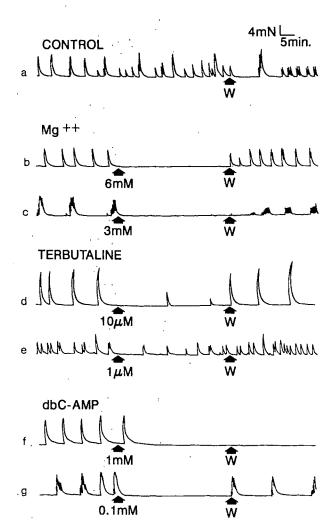


Fig. 1. Representative traces of effects of Mg++ (3 and 6 mmol/L), terbutaline (1 and 10 μmol/L), and dibutyryl cAMP (dbcAMP) (0.1 and 1 mmol/L) on spontaneous contractile activity in human myometrial strips. Traces (a to h) represent recordings from preparations from six different women. In each experiment with tissue from one subject all substances (Mg++, terbutaline, dibutyryl cAMP) were tested. The preparations were mounted horizontally between two small, L-shaped hooks in 5 ml organ baths (37° C), and isometric tension was recorded. W, Wash with Krebs-Ringer buffer. Time scale and scale of contractile response in millinewtons (10⁻⁸ newtons) are indicated at top right corner.

spective of the pattern of the activity (n = 6). The myometrial activity was rapidly reestablished when the bathing solution was replaced by buffer containing 1.2 mmol/L Mg $^{++}$ (Fig. 1, b and c). In five of six preparations tested, terbutaline (1 and 10 µmol/L) caused on average 75% and 90% reduction of the frequency of the contractions, respectively, and the amplitude was clearly diminished (Fig. 1, d and e). At 0.1 µmol/L, terbutaline only slightly (about 10% to 20%) reduced the amplitude, whereas the frequency of the contractions was unaffected (not shown). In only one of the

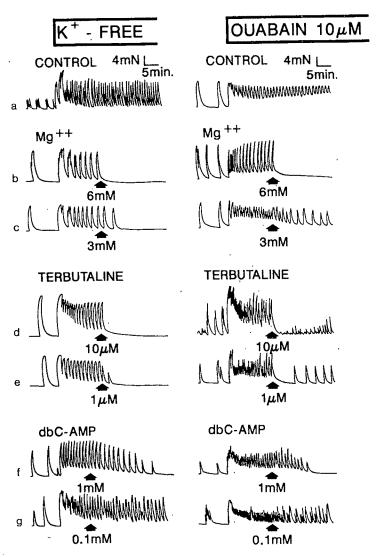


Fig. 2. Representative traces of contractile activity in human myometrial strips after replacement of Krebs-Ringer buffer by K⁺-free buffer (a, left) or addition of ouabain (10 μ mol/L) to the organ bath (a, right). Effects of Mg⁺⁺ (3 and 6 mmol/L), terbutaline (1 and 10 μ mol/L), and dibutyryl cAMP (dbcAMP) (0.1 and 1 mmol/L) on contractile activity induced by K⁺-free buffer or ouabain are shown in traces b to h. Traces a to h represent recordings from preparations from four different women. In each experiment with tissue from one subject all substances (Mg⁺⁺, terbutaline, dibutyryl cAMP) were tested. Time scale and scale of contractile response in millinewtons (10⁻³ newtons) are indicated at top right corner.

preparations terbutaline failed to affect the spontaneous contractile activity and the contractions induced by K^+ -free buffer or ouabain (see below). The myometrial activity was invariably restored after wash in Krebs-Ringer buffer (Fig. 1, d and e). Dibutyryl cAMP (1 mmol/L) irreversibly turned off spontaneous myometrial contractile activity (Fig. 1, f). Dibutyryl cAMP, 0.1 mmol/L, abolished the contractions in all instances (n = 6), but the activity was immediately restored after wash with Krebs-Ringer buffer (Fig. 1, g). In about 50% of the preparations the washing procedure induced a change of the pattern of the contractile activity as compared with the initial pattern. This was seen both in

control preparations and in preparations where Mg⁺⁺, terbutaline, or dibutyryl cAMP had been added and probably reflects a shift in pacemaker cell activity in the myometrial strips.

When normal Krebs-Ringer buffer (K⁺, 5.9 mmol/L) was substituted by a K⁺-free buffer, the contractile activity of the myometrial strips was considerably increased. The response consisted of transient tonic contractions with superimposed phasic contractions, continuing into a steady state with phasic contractile activity; sometimes with a minor elevation of basic tension (Fig. 2, left, n = 6). When a steady state had been achieved after initial stimulation of the con-

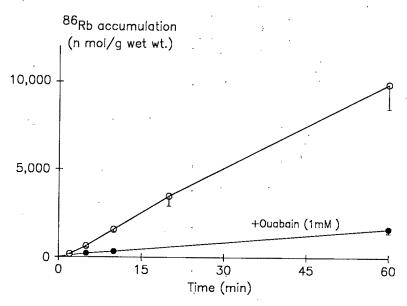


Fig. 3. Time course of ⁸⁶ Rb uptake in absence and presence of ouabain in human myometrial strips. Strips were equilibrated for 30 minutes at 30° C in Krebs-Ringer buffer and preincubated for 15 minutes in absence or presence of ouabain (1 mmol/L). This was followed by incubation lasting from 2 to 60 minutes in buffer containing ⁸⁶Rb (1 μCi/ml) without or with ouabain (1 mmol/L). Strips were then washed four times for 15 minutes each time at 0° C in Na⁺-free Tris-sucrose buffer. Thereafter, strips were blotted, weighed, homogenized in 2 ml trichloroacetic acid (0.3 mol/L), and taken for counting of ⁸⁶Rb activity by Cerenkov radiation. Each point represents means of 12 to 20 observations with *bars* indicating SE where this exceeds size of symbols. Data were obtained in five independent experiments with tissue from five different subjects. In each experiment at least two time points were measured.

tractile activity by K⁺-free buffer, as shown in Fig. 2, Mg⁺⁺ (3 and 6 mmol/L) in all instances and terbutaline (1 and 10 μ mol/L) in five of six preparations abolished the contractions (Fig. 2, left, b to e, n=6). However, terbutaline (0.1 μ mol/L) did not affect the activity induced by the K⁺-free buffer. Dibutyryl cAMP (1 mmol/L) also abolished the contractions induced by K⁺-free buffer, but the effect of this compound developed more slowly, and at 0.1 mmol/L dibutyryl cAMP had no effect (Fig. 2, left, f and g, n=6).

Ouabain (10 µmol/L) caused a contraction consisting of an initial peak and a second phase with maintained tension and superimposed phasic contractions (Fig. 2, right). Both the tonic component and the phasic contractions were invariably abolished by 6 mmol/L Mg++ (Fig. 2, right, b, n = 6). Mg^{++} , 3 mmol/L, reduced only the amplitude and the frequency of the phasic contractions (Fig. 2, right, c, n = 6). In all preparations but one, terbutaline (I and 10 µmol/L) transiently abolished the increased tone and the phasic contractions induced by ouabain (n = 6). Phasic contractile activity with varying amplitude and frequency then reappeared within 5 to 8 minutes (Fig. 2, right, d and e). At a concentration of 1 mmol/L dibutyryl cAMP gradually (within 10 minutes) abolished the contractile activity induced by ouabain in all preparations (n = 6), whereas 0.1 mmol/L had no significant effects (Fig. 2, right, f and g).

K⁺ uptake and efflux. The time course of the ⁸⁶Rb uptake in myometrial strips incubated in the absence or presence of ouabain (1 mmol/L) is shown in Fig. 3. In both cases 86Rb uptake approached a linear function of time. 86Rb uptake in the presence of ouabain amounted to 12% to 35% (60 and 5 minutes, respectively) of the total 86Rb uptake, indicating that the major part (65% to 88%) of the K+ uptake in this tissue is mediated through the sodium-potassium pump. The ouabain-suppressible K+ uptake (calculated from the 10-minute values) amounted to around 125 nmol/gm wet weight per minute. When ouabain-suppressible K+ uptake was measured with 42K, roughly the same value was obtained (Table I). This indicated that both 86Rb and 42K can be used to quantify the sodium-potassium pump-mediated K+ uptake. Furthermore, from Table I it can be seen that neither Mg++ (6 mmol/L) nor terbutaline (1 to 10 µmol/L) caused any significant changes in the ouabain-suppressible 42K or 86Rb uptake during an incubation period of 10 minutes. This implied that neither Mg++ nor terbutaline had any effect on the sodium-potassium pump-mediated K⁺ uptake. However, in the initial phase the 86Rb uptake as expressed per minute was somewhat reduced, presumably reflecting diffusional delay for the access of isotope to interstitial space (see also Table II).

The study of Daniel et al. 18 suggested that the effect of isoproterenol on 12 K uptake in rat myometrium be-

Table I. Effects of Mg⁺⁺ and terbutaline on ouabain-suppressible ⁴²K and ⁸⁶Rb uptake in human pregnant myometrial strips

	Ouabain-suppressible ¹² K uptake (nmol/gm wet wt/min)	Ouabain-suppressible ⁸⁶ Rb uptake (nmol/gm wet wt/min)		
Control	$109 \pm 22 \ (n = 12)$	$90 \pm 19 (n = 12)$		
Mg ⁺⁺ (6 mmol/L) Terbutaline	$116 \pm 26 \ (n = 12)$	$85 \pm 10 \ (n = 12)$		
l μmol/L	$109 \pm 15 \ (n=4)$	ND		
10 μmol/L	$139 \pm 36 \ (n=8)$	$86 \pm 15 (n = 12)$		

Values are mean \pm SE with the number of observations in parentheses. Myometrial strips weighing around 20 mg were prepared. ⁴²K and ⁸⁶Rb uptake was measured in the absence or presence of ouabain (1 μ mol) during 10 minutes of incubation followed by washout four times for 15 minutes each at 0° C as described in legend to Fig. 3. Tissue was obtained from four subjects. From each subject a group of four control specimens was tested against either Mg⁺⁺ (6 μ mol/L) or terbutaline (1 or 10 μ mol/L). None of the effects observed gained statistical significance (p > 0.10). ND, Not determined.

Table II. Effects of terbutaline on time course of ⁸⁶Rb uptake in absence and presence of ouabain in human pregnant myometrial strips

	Total 86 Rb uptake (nmol/gm wet wt/min)	Ouabain-suppressible ⁸⁶ Rb uptake (nmol/gm wet wt/min)
2 min		
Control	$103 \pm 7 (n = 8)$	ND
Terbutaline (10 µmol/L)	$132 \pm 13 \ (n=8)$	ND .
5 min	, .	
Control	$159 \pm 13 \ (n = 8)$	$116 \pm 14 \ (n = 8)$
Terbutaline (10 µmol/L)	$161 \pm 18 \ (n = 8)$	$125 \pm 18 \ (n = 8)$
10 min	· · ·	,
Control	$198 \pm 28 \ (n=8)$	$166 \pm 29 (n = 8)$
Terbutaline (10 µmol/L)	$183 \pm 21 \ (n = 8)$	$155 \pm 21 \ (n = 8)$
60 min	, ,	, ,
Control	$209 \pm 21 (n = 8)$	$184 \pm 21 \ (n = 8)$
Terbutaline (10 µmol/L)	$197 \pm 14 \ (n = 8)$	$173 \pm 14 \ (n = 8)$

Values are mean \pm SE with the number of observations in parentheses. Strips weighing around 20 mg were prepared. ⁸⁶Rb uptake was measured in the absence or presence of ouabain (1 mmol/L) with incubations lasting between 2 and 60 minutes followed by washout four times for 15 minutes each at 0° C as described in legend to Fig. 3. Tissue was obtained from two subjects, and the results are pooled from experiments on tissue specimens from both subjects. None of the effects observed gained statistical significance (p > 0.05). ND, Not determined.

came more evident after 60 minutes of incubation. On the other hand, the effect of isoproterenol on active sodium-potassium transport in another smooth muscle preparation¹⁵ was maximal within 5 minutes of exposure. However, in our experiments there was no effect of terbutaline (10 µmol/L) on ⁸⁶Rb uptake in the absence or presence of ouabain, as measured during incubation periods of 2, 5, 10, or 60 minutes (Table II).

It has been proposed that β_2 -adrenergic receptor activation leads to opening of K⁺ channels, which could induce hyperpolarization and ensuing relaxation³³; therefore the effect of terbutaline (10 μ mol/L) on the fractional loss of ⁴²K from the myometrial strips also was assessed, but no significant change was observed (n = 3). Mg⁺⁺ (6 mmol/L) produced no significant effect (n = 3), but ouabain (1 mmol/L) increased the fractional loss of ⁴²K by 106% within 15 minutes (p < 0.001, n = 3) (data not shown). From the fractional loss of K⁺ at rest and the K⁺ content by the end of the experiment in the control strips, the absolute

rate of K⁺ efflux could be calculated. This amounted to $0.0038~\text{min}^{-1} \times 40.4~\mu\text{mol/gm}$ wet weight = 154 nmol/gm wet weight per minute (i.e., a value not far below the total K⁺ uptake, indicating that the strips were in steady state).

Table III shows the results of experiments where agents that are known to stimulate K⁺ uptake in skeletal muscle (insulin, dibutyryl cAMP)^{31, 34} or to produce a rise in cellular cyclic adenosine 5′-monophosphate (forskolin)³⁵ were tested. None of the agents, insulin (100 mU/ml), forskolin (10 μmol/L), or dibutyryl cAMP (1 mmol/L), significantly increased ⁸⁶Rb uptake.

Several of the agents known to stimulate active sodium-potassium transport in skeletal muscle failed to stimulate ⁸⁶Rb uptake; therefore it was important to examine whether the sodium-potassium pump of the myometrium could be stimulated by Na⁺ loading. In Na⁺-loaded strips exposed to K⁺-rich buffer (151.2 mmol/L K⁺), the ouabain-suppressible ⁸⁶Rb uptake amounted to 525 ± 136 (n = 12) nmol/gm wet weight per minute (i.e., a fivefold higher rate than the basal values given in Table I).

To see whether K+ uptake varied in the different parts of the myometrium, 86Rb uptake was compared in strips prepared from biopsy specimens of the isthmic and fundus region. The basal rate of 86Rb uptake was similar in both parts of the myometrium, and no differences concerning the effects of Mg++ (6 mmol/L), terbutaline (10 µmol/L), or dibutyryl cAMP (1 mmol/L) on the 86Rb uptake could be detected (data not shown).

Comment

This study was undertaken to determine whether the effects of Mg++ and the β_2 -adrenergic receptor agonist terbutaline on the contractile performance of human pregnant myometrium could be related to effects on sodium-potassium-adenosine triphosphatase activity.

Although with some variation between tissue from different subjects, the amplitudes and frequencies of the spontaneous contractile activity of the myometrial strips used in this study were similar to those previously reported.7-10, 28

The results show that terbutaline, in a concentrationdependent way, inhibited the spontaneous contractions up to complete suppression of the activity at a concentration of 10 µmol/L. The exact mechanism for the action of the β₂-adrenergic receptor agonists has yet to be established, but it is generally accepted that the receptor occupation results in activation of adenylate cyclase, which increases the intracellular content of cyclic adenosine 5'-monophosphate. However, in rat myometrium it was found that the relaxation resulting from activation of \$\beta_2\$-adrenergic receptors was mediated through cyclic adenosine 5'-monophosphate-dependent and cyclic adenosine 5'-monophosphate-independent mechanisms.34 Still, the relaxant effect of β2adrenergic receptor agonists can be attributed partly to the intracellular actions of cyclic adenosine 5'-monophosphate, and in human myometrium a direct correlation between the myometrial level of cyclic adenosine 5'-monophosphate and the relaxant effects of β2adrenergic receptor agonist has been demonstrated.7 Our results support this view, because both terbutaline and dibutyryl cAMP abolished the spontaneous contractions of the myometrial strips.

It has been proposed that the increased level of cyclic adenosine 5'-monophosphate caused by the β2-adrenergic receptor agonists stimulate active sodium-potassium transport,12-14, 16, 17 leading either to increased Ca++ extrusion by the Na+-Ca++ exchange mechanism8 or to decreased Ca++ entry through voltage-operated Ca++ channels because of the hyperpolarization of the cell membrane.17 Our results obtained with human myometrium, however, strongly argue against this view.

Table III. Effects of insulin, forskolin, and dibutyryl cAMP on 86Rb uptake in human pregnant myometrial strips

	Total ⁸⁶ Rb uptake (nmol/gm wet wt/min)
No additions	$226 \pm 12 (n = 12)$
Insulin (100 mU/ml)	$209 \pm 14 (n = 8)$
Forskolin (10 µmol/L)	$203 \pm 18 (n = 8)$
Dibutyryl cAMP (1 mmol/L)	$229 \pm 17 \ (n = 8)$

Values are mean ± SE with number of observations in parentheses. Strips weighing around 20 mg were prepared from isthmic region of the uterus (one subject). 86Rb uptake was measured with 10 to 20 minutes of incubation followed by washout four times for 15 minutes each at 0° C as described in legend to Fig. 3.

Ouabain and K+-free solution increased myometrial activity and tension presumably because of depolarization of the strips after inhibition of the sodium-potassium pump.36 In spite of this inhibition or blockade, both dibutyryl cAMP and terbutaline were able to abolish the contractile responses. Furthermore, both failed to stimulate the ouabain-suppressible 86Rb and 42K uptake in isolated myometrial strips.

None of the agents that are known to stimulate active sodium-potassium transport in skeletal muscle (catecholamines, insulin, dibutyryl cAMP) had any detectable effect on the ouabain-suppressible 86Rb or 42K uptake in the human myometrium. However, when the myometrial strips were loaded with Na+ and exposed to high K+, a fivefold stimulation of the active K+ uptake was observed. These results are in good agreement with those of Sanbourn,21 who found that momensin (which increases intracellular Na+) but not isoproterenol could stimulate 86Rb uptake in cultured rat myometrial cells. On the basis of our previous data on the total concentration of sodium-potassium pumps in the human myometrium (72 pmol/gm wet weight²⁹), an estimated maximum transport rate of the sodium-potassium pump of 8900 K+ ions per minute at 30° C,30 and the transport rates measured here, it could be calculated that the sodium-potassium pump in the Na+loaded strips reached around 80% of its maximum rate $(525 \times 100/0.072 \times 8900 = 82\%)$. However, under basal conditions the pumps are functioning at only 15% to 20% of the maximum transport rate. The variation observed in the measurements of 86Rb and 42K uptake is probably caused by interindividual differences in the concentration of sodium-potassium pumps, intracellular Na⁺, or both.

For isolated rat myometrium it was suggested that the β2-adrenergic receptor agonist-induced increase in cyclic adenosine 5'-monophosphate was responsible for the observed hyperpolarization of the cell membrane, and this was proposed to occur through increased K+

conductance.33 These authors further demonstrated that the hyperpolarization of the cell membrane was absent in Ca++-free medium and suggested that part of the hyperpolarization might be due to a stimulation of active Ca⁺⁺ efflux by cAMP. This hypothesis was supported by the results of Fortier et al.,37 who demonstrated that isoproterenol increased ¹⁵Ca⁺⁺ efflux in rat myometrial cell culture and that this effect was mediated by cAMP. Our results do not allow for any conclusion as to whether this is the mechanism of action of terbutaline and cAMP in the intact human myometrium as well. However, both terbutaline and Mg++ failed to increase the fractional loss of 42K from the myometrial strips, indicating that mechanisms other than increased K+ conductance are involved in the observed hyperpolarization of the cell membrane.38

Previous studies on isolated human nonpregnant myometrium²⁷ have tested only inhibitory effects of the Mg⁺⁺ at concentrations exceedingly higher (12 to 24 mmol/L) than therapeutic levels. Our results showed that Mg⁺⁺, also at concentrations within the therapeutic range of 2 to 4 mmol/L,³⁸ completely abolished the spontaneous contractions of the myometrial strips from pregnant women. Furthermore, Mg⁺⁺ abolished the contractions induced by ouabain and K⁺-free buffer and failed to stimulate ⁸⁶Rb and ⁴²K uptake in the myometrial strips. These data therefore suggest that the relaxant effects of Mg⁺⁺ cannot be attributed to increased sodium-potassium-adenosine triphosphatase activity.

Our study does not allow for differentiation between the mechanisms of relaxation of terbutaline and Mg++. There is no evidence, however, that the relaxant effects of Mg++ on smooth muscle should be mediated by an increase in intracellular cAMP. On the contrary, Sjögren and Edvinsson²⁴ found in cat vascular preparations that Mg++ interfered with Ca++ release from intracellular depots without involvement of cAMP. There is evidence that Mg++ also antagonizes the effects of Ca++ at other sites.39 Thus in human uteroplacental vasculature Mg++ was found to inhibit the responses to extracellular Ca++, probably by interfering with Ca++ influx through voltage-operated Ca++ channels.25, 26 In addition, Mg++ inhibited both resting and K+-stimulated 45Ca influx in isolated human nonpregnant myometrial strips.27

In summary, the results of this study show that the relaxant effects of Mg⁺⁺ and terbutaline in the human pregnant myometrium persist in the absence of K⁺ or in the presence of ouabain, indicating that these effects are not due to stimulation of the sodium-potassium pump. This conclusion was further confirmed by measurements of sodium-potassium pump-mediated K⁺ uptake in the same preparations. Other possible mechanisms for the relaxant properties of Mg⁺⁺ and terbutaline are currently being investigated.

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Fungal morphology after treatment with itraconazole as a single oral dose in experimental vaginal candidosis in rats

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The therapeutic effect of a single, oral dose of itraconazole was studied in rats inoculated intravaginally with Candida albicans and in which an established vaginal infection was present. We used light microscopy, transmission and scanning electron microscopy to document the structural alterations in the 3 days after treatment. The most important observations include the speed (within 24 hours) with which itraconazole inhibits the further penetration of the fungus into the vaginal squamous epithelium, the ability of the drug to reach and structurally alter intracellularly located fungal elements, and the prolonged drug effect of a single dose leading to complete eradication of the fungus from the vagina within 3 days. (AM J OBSTET GYNECOL 1991;165:1552-7.)

Key words: Itraconazole, single oral dose; vaginal candidosis, rat; light microscopy, transmission electron microscopy, scanning electron miscroscopy

Itraconazole is an orally active triazole antifungal. It has performed well in the treatment of experimental vaginal candidosis in animals1-5 and in clinical trials with vaginal candidosis. 6-8 In both instances the total antifungal dose administered has proved to be more important than the length of therapy (usually 1, 2, or 3 days).3,7 This observation has led to shorter treatment courses with higher antifungal doses and finally to 1day therapies, resulting in superior patient compliance compared with the long courses of oral or topical antifungals.9 In experimental and clinical studies cure rates mostly have been based on both clinical cure and culture of vaginal samples. Although these methods are valuable to assess therapeutic results, a clear picture of the interaction of the antifungal drug with the fungus in situ cannot be obtained. In this respect ultrastructural studies of the morphologic features and distribution of the fungus within the vaginal epithelial layers may offer important, complementary information on the drug-fungus interaction, especially in the early phase after treatment. To study the ability of itraconazole, given as a single oral dose, to induce ultrastructural fungal damage, and finally to eradicate Candida albicans from the vagina, we used light microscopy and transmission and scanning electron microscopy in rat vaginal candidosis, which is a well-established model for the evaluation of antifungals1, 8-5, 10-12 and which

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shares important features with those described for human vaginal infections.9

Material and methods

Female Wistar rats weighing 100 gm underwent oophorectomy and hysterectomy. Eighteen days later, 100 µg of estradiol undecylate in sesame oil was injected subcutaneously into each rat. The induced pseudoestrus was checked by microscopic examinations of vaginal smears. Only animals in pseudoestrus were inoculated intravaginally with 8 × 10⁵ blastospores of C. albicans (strain ATCC 44858). Curative treatment with placebo or with itraconazole (10 or 20 mg/kg) was given as a single dose by gavage on the fourth day after inoculation. Each treatment group consisted of six animals. Two control rats, untreated but infected, were killed on the fourth day after infection, and two rats of each treatment group were killed at 24, 48, and at 72 hours after treatment. The vagina was removed and fixed as a unit in 3% glutaraldehyde buffered to pH 7.4 with 0.1 mol/L sodium cacodylate at room temperature for 48 hours. The vagina was then divided into different parts (separate pieces for light and transmission electron microscopy and for scanning electron microscopy) and washed in the same buffer to which 0.22 mol/L sucrose was added. The specimens were then postfixed for 1 hour in 2% osmium tetroxide buffered to pH 7.4 with 0.05 mol/L veronal acetate. After a brief wash in the buffer, they were impregnated with 0.5% uranium acetate in veronal acetate buffer (pH 5.2) for 40 minutes and subsequently rinsed in the same buffer. After dehydration in a graded series of ethanol, they were routinely embedded in epon. Semithin sections were made for light microscopic exami-

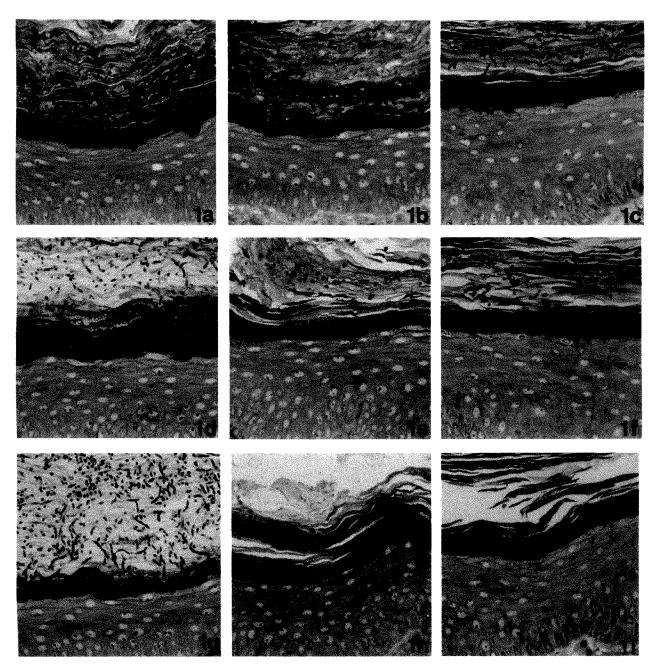


Fig. 1. Light microscopy of rat vaginal epithelium illustrating progression of C. albicans infection in squamous cell layers under placebo treatment and curative effect of itraconazole treatment. (Original magnification ×250.) a to c, 24 Hours after oral treatment with placebo (a), itraconazole 10 mg/kg (b), and itraconazole 20 mg/kg (c). Fungus-free zone of 10 to 20 squamous cell layers adjacent to nucleated cell layers can be seen in b and c. d to f, 48 Hours after oral treatment with placebo (d), itraconazole 10 mg/kg (e), and itraconazole 20 mg/kg (f). Effect of treatment after 48 hours is comparable with that after 24 hours. g to i, 72 hours after oral treatment with placebo (g), itraconazole 10 mg/kg (h), and itraconazole 20 mg/kg (i). In g squamous epithelium is severely infected, whereas in h only a few fungi are found in superficial cell layers. In i treatment has resulted in complete eradication of fungus from vaginal epithelium.

nation and stained with toluidine blue (0.1%, pH 11.1). Ultrathin sections were counterstained with uranium acetate and lead citrate and examined in a Philips EM 300 transmission electron microscope. For scanning electron microscopy examination, the same fixation procedure was followed. After ethanol dehydration, the specimens were passed through a gradient of ethanol-acetone mixtures until a 100% acetone solution

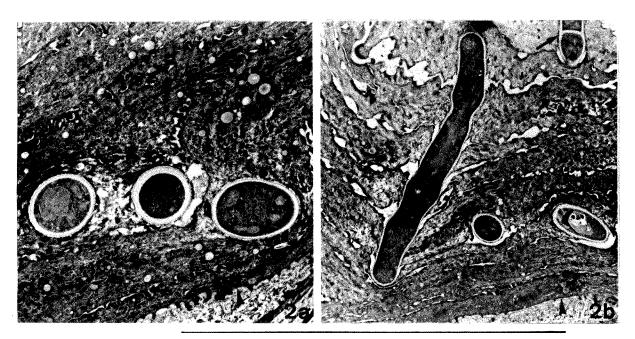


Fig. 2. Transmission electron microscopy of *C. albicans* found in deepest layers of vaginal squamous epithelium of placebo-treated rats. *Arrowheads* point at line that separates squamous (top) from nucleated (bottom) epithelial cell layers. a Represents transverse cuts through three Candida. Note viable cytoplasmic content and cell wall of uniform thickness within each element. (Original magnification, ×8750.) b Represents longitudinal section of Candida hypha that is penetrating squamous epithelium. *Arrow* in upper right corner points at septum of another hypha. (Original magnification, ×3895.)

was reached. They were dried at the critical point, sputtered with a layer of gold, and examined in a Philips PSEM 500 microscope.

The experiments were conducted in accordance with the principles and standards as set forth in the Guide for the Care and Use of Laboratory Animals, the Johnson & Johnson Policy on the Humane Care and Use of Experimental Animals, and the International Guiding Principles for Biomedical Research Involving Animals.

Results

Light, scanning electron, and transmission electron microscopy of the vaginal wall revealed that 4 days after inoculation of yeasts, the vaginal epithelium still presented a picture of pseudoestrus, characterized by marked hyperkeratosis and pronounced exfoliation of the horny layer. The entire squamous cell layer was colonized by fungal elements, both yeast cells and filamentous forms, but the fungi did not penetrate deeper than the keratinized epithelium. Transmission and scanning electron microscopy confirmed the presence of C. albicans within the cytoplasm of epithelial squamous cells, and interspersed between epithelial cells, fungal elements together with bacteria were abundant. Inflammatory cells were not present. Transmission electron microscopy of cross sections of fungal elements, in most instances, revealed a normal ultrastructure and only occasional cells presented a degenerated cytoplasm. Thus, at the moment oral therapy

was given, an established vaginal fungal infection was present. One day later the infection had further increased in severity in the placebo-treated animals, as evidenced in vaginal cross sections by the increased number of fungal elements on light, transmission electron, and scanning electron microscopy, but penetration into the nucleated epithelial cell layers did not take place (Fig. 1, a). However, in animals treated with itraconazole (10 and 20 mg/kg), a yeast- and myceliumfree zone of squamous epithelium adjacent to the nucleated cell layers was found (Fig. 1, b and c). The fungus-free zone comprised between 10 and 20 squamous cell layers. On transmission electron microscopy, fungal elements in all locations from the lumen to the deepest site within the horny cell layers showed cell wall alterations typical of azole treatment (Fig. 2, a and b, and Fig. 3, a and b). These changes, which also were noted in fungal elements located intracellularly, consist of an irregularly thickened cell wall containing phospholipid-like vesicles. Such vesicles also accumulate between the plasma membrane and the cell wall. Necrosis of the intracellular contents, together with a decreased number of fungal elements, was a further difference noted on comparison with the placebo-treated animals, although these were less objectively evident. No clear difference was seen day 1 or 2 after treatment between animals treated with placebo or itraconazole (10 or 20 mg/kg) (Fig. 1, b, c, e, and f).

Three days after treatment, however, a substantial

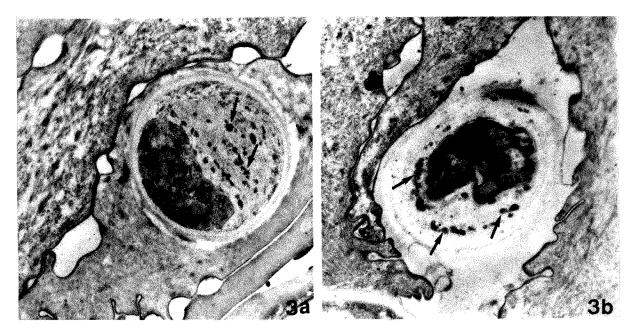


Fig. 3. Transmission electron microscopy of C. albicans in squamous vaginal epithelium of itraconazole (10 mg/kg)-treated rats, 24 hours after treatment, illustrating early changes, typical of azole treatment: electron-dense inclusions (arrows) in abnormally thickened cell wall. Changes are seen in intracellularly (a) and in extracellularly (b) located fungal elements. (a, Original magnification \times 18,600. **b,** Original magnification \times 16,200.)

difference existed between the rats dosed with 10 and 20 mg/kg. Where the infection was still present in the upper squamous cell layers of the 10 mg/kg treated animals, with numerous necrotic fungal elements demonstrating the cell wall changes described above, the 20 mg/kg dose had resulted in a complete eradication of the fungus from the vaginal epithelium and the intraluminal cellular debris (Fig. 1, g to i, and Fig. 4, a to c).

A bacterial flora was present in all vaginal specimens without exception and on no occasion seemed to be influenced by the therapy. In some specimens neutrophils, situated in the upper epithelial layers and in the vaginal lumen, were seen to engulf bacteria avidly, but phagocytosis of fungal elements was not seen in any of the treatment groups.

Comment

The findings of this study indicate that, in a wellestablished vaginal C. albicans infection in rats, a single dose of itraconazole is able to induce ultrastructural cell wall changes in fungal elements located within the vaginal epithelium, even in those that are located intracellularly, and this is within 24 hours after a single-dose therapy. The changes seen, inclusions in the cell wall and between the cell wall and the plasma membrane, have been reported previously for itraconazole and other azole antifungals in vitro and in vivo after topical and oral treatment and may be considered a consequence of azole treatment because they never occur in

spontaneously degenerating cells.2.13-15 They are to be considered as the morphologic counterpart of the inhibition of ergosterol biosynthesis in the fungal cell, a mechanism of action common to all azole antifungals. 16-18 The appearance of cell wall alterations parallels the observation that in the 24 hours after therapy the fungus is no longer able to invade the newly formed hyperkeratotic cell layers. This observation is consistent with the data on the interaction of itraconazole with C. albicans in vitro, 2, 19, 20 which demonstrate that the drug is able to inhibit the transformation of the yeast form into the filamentous form at concentrations that can be reached in plasma and tissue in man and animals after oral administration.21.22 The flexible interconversion between blastospores and filamentous forms and vice versa has been implicated as an important factor and maybe even as the determining factor in the pathogenicity of C. albicans in natural infections and is considered to be more important than the role played by either form alone.9 Intracellular hyphal elements have been described by others,23-25 and it is possible that they provide a means of escaping host defense systems. 15 Two possible explanations for the relapse sometimes seen after antifungal therapy have been either that antifungal drugs with poor lipophilicity and limited tissue penetration do not reach these intracellular fungal cells or that the latter are not metabolically active and therefore are not affected by the antifungal action of the drug. In this respect the observation that intracellular fungal elements do seem to be metabolically active and 1556 Jansen et al.

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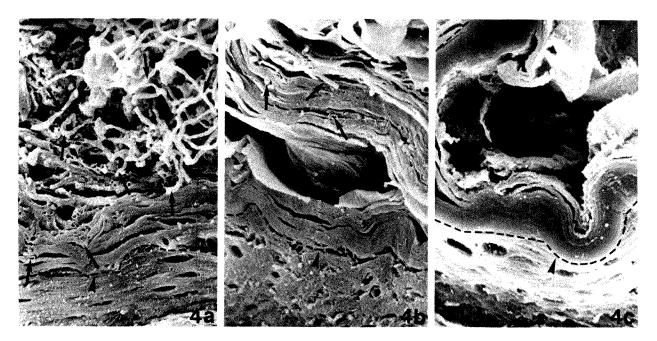


Fig. 4. Scanning electron microscopy of rat vaginal epithelium at 72 hours after single oral treatment, illustrating extensive colonization of squamous epithelium by *C. albicans* hyphae after placebo treatment (a). Treatment with 10 mg/kg itraconazole has inhibited further hyphal invasion of newly formed squamous cell layers (b), whereas 20 mg/kg itraconazole treatment has eradicated yeast from vaginal wall (c). *Arrowheads* point at *dotted line*, separating squamous from nucleated epithelial cell layers, whereas *arrows* point at *C. albicans* hyphae. (Original magnification ×500.)

that they are within reach of the action of itraconazole may well be an explanation for the unusually low relapse rates observed after use of this compound in the treatment of vaginal candidosis. The typical alterations of fungal morphologic features within 24 hours of therapy illustrate that the fast action of itraconazole is a direct result of a drug-*Candida* interaction and not the result of an indirect effect of the drug on the turnover of the vaginal epithelium, an action that has been reported to contribute to the overall therapeutic effect of ketoconazole in the same animal model. This observation further illustrates the importance of evaluating the effect of antifungal drugs on the host-pathogen interaction in situ.

Transverse sections of the vaginal squamous epithelium made in the 3 days after treatment clearly illustrate the depth of the infection and the effect of a fungistatic treatment on it. At each moment the aspect of the infected vaginal epithelium is determined by the speed with which the fungus penetrates the newly formed squamous cell layers and the speed with which these new cell layers are formed. Both itraconazole doses were equally effective in reaching and inhibiting the most deeply located fungal elements, but complete eradication of yeasts from the vagina within 3 days was obtained only with the 20 mg/kg dose. This observation is in agreement with that of Sobel and Muller,³ who reported that complete mycologic cure could be ob-

tained with a single dose of 25 mg/kg in the same animal model. The same efficacy could be obtained by one of us27 with a 3-day regimen of itraconazole 2.5 mg/kg/day, which illustrates the importance of the dose administered in relation to the duration of therapy, a relation that also has been confirmed in patients by Cauwenbergh.7 The prolonged action of itraconazole after a single dose can be explained by the drug's lipophilic properties, which guarantee sustained tissue levels for several days at the infection site. Determination of drug concentrations in human vaginal tissue after a single oral dose of 200 mg and in rat vaginal fluid after a single oral dose of 10 mg/kg has confirmed that inhibitory concentrations can be found for at least 3 days.^{3, 5, 28} The fact that these levels have to be high enough for a critical period of time to obtain complete eradication is well illustrated in this study and agrees with the observations of Sobel and Muller³ that suboptimal single doses do not result in mycologic cure.

In healthy rats polymorphonuclear leukocytes have been noticed in the vaginal lumen and in the upper epithelial layers during late metestrus and particularly during diestrus.²⁰ When *C. albicans* blastospores were introduced into the vagina of rats and mice during diestrus, phagocytosis of spores was observed.^{30, 31} All attempts however to induce an infection of the squamous epithelium at this stage of the estrus cycle have proved to be unsuccessful. In rats and mice with vaginal

candidosis artificially induced during pseudoestrus and in spontaneous vaginal *Candida* infections in women, the presence of polymorphonuclear leukocytes, although not consistently, was noticed. ³⁰⁻³² Ingestion of *Candida* by these polymorphonuclear leukocytes, however, was not reported.

Although phagocytic cells are believed to be major components of the host defense against deep-seated *Candida* infections, clinical and experimental observations reported so far support the current opinion that phagocytic cells are less important in the defense against *Candida* in superficial sites, such as the mouth, the skin, and the vagina.³³ In these sites defense probably depends critically on a multiplicity of host defense factors, as it does in any part of the body.

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Role of endogenous atrial natriuretic factor in the regulation of fetal cardiovascular and renal function

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The purpose of the present study was to determine whether endogenous atrial natriuretic factor participates in the maintenance of normal vascular pressure and renal function in ovine fetuses at 128 to 130 days' gestation. Circulating atrial natriuretic factor in the fetus was immunoneutralized by an intravenous bolus injection of an atrial natriuretic factor antiserum at a dilution of 1:2000 (low dose, n = 7) or 1:400 (high dose, n=6). In the high-dose group, plasma atrial natriuretic factor concentration was significantly reduced by 65 \pm 14 pg/ml from basal levels of 165 \pm 12 pg/ml within 10 minutes and remained reduced for the 90-minute period after the injection. Fetal arterial pressure acutely and transiently decreaased, but at 50 minutes arterial pressure increased and was elevated for the remainder of the experiment. Urine flow and urinary excretion rates of sodium, potassium, and chloride were reduced within 10 minutes after the injection. Urine flow rate was suppressed for as long as plasma atrial natriuretic factor concentrations were reduced. Fetuses in the low-dose and control groups showed no significant change in cardiovascular or renal function. In response to atrial natriuretic factor antiserum injection, plasma angiotensin II concentrations were increased, whereas plasma arginine vasopressin concentrations were unchanged. These results suggest that endogenous atrial natriuretic factor is involved in the maintenance of arterial pressure and urinary excretion in the ovine fetus. (AM J OBSTET GYNECOL 1991;165:1558-67.)

Key words: Atrial natriuretic factor, fetus, antiserum, urine flow, vascular pressure, angiotensin II, arginine vasopressin

In the mammalian fetus, atrial natriuretic factor (ANF) has been implicated in the regulation of blood pressure and fluid volume. Administration of ANF into the fetal circulation decreases arterial pressure and blood volume, while having little effect on venous pressure or heart rate. The renal effects of ANF are largely similar to those in the adult, in that it increases fetal urine flow rate, glomerular filtration rate, and enhances urinary electrolyte excretions.2.3 However, these effects occur only at high ANF concentrations and appear to be gestational-age dependent.4 Contrary to that in the adult, the interaction of ANF with other endocrine systems in the fetus is minimal, except at supraphysiologic concentrations when arginine vasopressin and plasma renin activities are increased in response to ANF infusion. 5,4 These observations suggest that ANF may not be playing a significant role in the regulation of vascular pressures or fluid dynamics in the fetus. However, ANF concentrations in the fetal circulation are higher than

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those in the adult,4-6 and physiologic manipulations of the fetal cardiovascular system can significantly alter fetal plasma ANF concentrations. For example, expansion of the fetal vascular volume7 or fetal hyperosmolality8 increases plasma ANF levels, whereas reduction in fetal blood volume decreases circulating ANF concentrations.9 Furthermore, fetal hypoxia elevates plasma ANF levels.10 Therefore, to document in the fetus the contribution of endogenous ANF to the physiologic maintenance of blood pressures, urine flow, and electrolyte excretion and its interaction with other hormones, this study was undertaken to investigate the cardiovascular, renal, and endocrine effects of blockade of endogenous ANF action. Since a physiologic antagonist to ANF is currently not available, an antiserum was used for these studies, to immunoneutralize endogenous ANF in the fetal circulation.

Methods

Eleven pregnant sheep with singleton fetuses were used for these studies. The experimental protocol was approved by the University committee on animal research. We followed the guidelines of the University and the National Institutes of Health for the care and use of these animals. With the animals under gas inhalation anesthesia (1% to 2% halothane in oxygen), chronic vascular catheters were implanted in the fetal descending aorta and inferior vena cava at the level of

the diaphragm. Additional catheters were placed in the urinary bladder and in the amniotic cavity using procedures previously described.¹¹ Prophylactic antibiotics were administered to the ewe and into the amniotic fluid for the first 5 postsurgical days.

Experiments were carried out at least 5 days after surgery. The gestational ages of the fetuses at the time of experimentation were 128 to 139 days (term 145 to 150 days). Four fetuses were experimented on once, and seven were experimented on twice, with 48 hours allowed for recovery between experiments. During the experiment, arterial pressure, venous pressure, heart rate, amniotic fluid pressure, and urine flow rate were continuously monitored on a polygraph recorder, and data were stored with an on-line computer.12 The fetal vascular pressures were continuously corrected with the on-line computer, with amniotic fluid pressure used as the zero pressure reference. For data analysis of vascular pressures, heart rate, and urine flow rate. 2-minute averages of the on-line data were taken and stored in a file for statistical evaluation.

After a 30-minute control period, an ANF antiserum was injected intravenously as a bolus into the fetal circulation. This was followed by a 90-minute observation period. The antiserum was generated against the carboxyl terminal of human ANF₁₋₂₈ (Peninsula Laboratories, Belmont, Calif.). We have successfully used this antiserum for the measurement of ANF concentrations in the pregnant sheep and their fetuses.5 The antiserum recognizes ovine ANF and, when used at a dilution of 1:25,000 for radioimmunoassay (RIA), it has a sensitivity of 3 pg of human ANF₁₋₂₈. One group of fetuses (low dose, n = 7) was given the antiserum at a dilution of 1:2000 in 2 ml of 1% normal rabbit serum diluted in phosphate-buffered saline solution. A second group of fetuses (high dose, n = 6) was given the antiserum at a dilution of 1:400 in 2 ml of 1% normal rabbit serum. A separate group of fetuses were used as controls (n = 5) and were given 2 ml of 1% normal rabbit serum.

Samples of fetal blood at 3.5 ml each and urine at 1 ml each were taken at 10 minutes after the start of the control period and at 15-minute intervals thereafter. The fetal blood removed by blood sampling was replaced by an equal volume of heparinized maternal blood. In the blood samples, blood gases (IL 1302 blood gas analyzer, Instrumentation Laboratory), hematocrit (in triplicate), plasma protein concentration (American Optical, TS meter), and plasma ANF, arginine vasopressin, and angiotensin II concentrations were measured. In the urine samples, osmolality (Advanced Diagnostic Osmometer 3D2) and electrolyte concentrations (Nova 5 + 5 electrolyte analyzer) were measured. Plasma ANF concentrations were determined by RIA with procedures previously described⁵ with the follow-

ing exception: The ANF concentration in the samples was measured by a direct assay without the extraction step. This was necessary because the ANF antibody, when injected into the fetus, became bound to the hormone, thus reducing the concentration of free ANF in the circulation. Extraction of these plasma samples with the Sep-Pak procedure would dissociate the antibody from the ANF, thereby freeing the ANF for subsequent RIA. This would result in measurement of total ANF in the plasma instead of free ANF left unbound by the injected antiserum. By using the plasma directly for RIA without extraction, the concentration of free ANF in the plasma not bound by the injected antiserum could be obtained. In a previous study in the rat a similar direct assay procedure was used to determine the level of free ANF in the circulation after antibody administration.13 We have validated this method by the addition of the antiserum to a pool of fetal plasma before subjecting to direct RIA and obtained antiserum dilution-dependent reductions of ANF concentration in the plasma. Furthermore, when the fetal plasma pool (without antiserum addition) was measured by the direct method, as well as after extraction, the mean value obtained with the direct method was 9% greater than with the extracted method. There were no statistically significant differences between the values obtained by the two methods. Plasma concentrations of arginine vasopressin and angiotensin II were determined by RIA after extraction, as has been reported previously.¹⁴

The data are presented as the mean \pm SE. The change in blood volume at a given time was calculated as a percent of control using the mean hematocrit values.15 To explore changes with time in a given variable, a two-factor analysis of variance was used, with time and animal being the two factors. If a significant F value was obtained, individual group means were compared with Fisher's least-significant-difference test. In some instances, when the intraanimal variability was large, a nonparametric analysis of variance was used. (Friedman's nonparametric test, which was the equivalent of a two-factor analysis of variance, with time and animal being the two factors, calculated a value for χ^2 .) Data were considered statistically significant if p < 0.05. Urinary excretion rate of each of the electrolytes—sodium, potassium, and chloride—was calculated as the product of urine flow rate and urinary concentration of the electrolyte.

Resuits

Basal conditions. For the fetuses in the control, low-dose, and high-dose groups, the gestational age at the time of experimentation and the control values during basal conditions are given in Table 1. There was no significant difference in any of these variables among the three groups of animals studied.

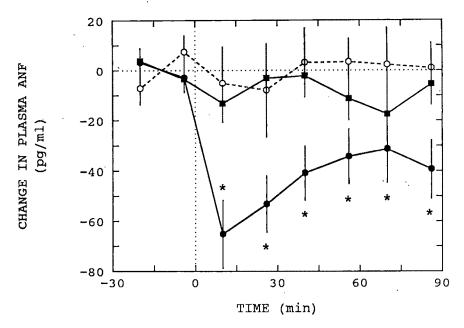


Fig. 1. Changes in fetal plasma ANF concentration (mean \pm SE) after ANF antiserum injection at 30 minutes in low-dose (solid squares), high-dose (solid circles), and control (open circles) groups. Asterisk, p < 0.05 compared with control values during first 30 minutes of experiment. Dotted line, Time of ANF antiserum injection.

Table I. Basal values before antiserum injection in three groups of fetuses

	$\begin{array}{c} Control\\ (n=5) \end{array}$	$ Low \\ (n = 7) $	High (n = 6)
Gestational age (days)	134 ± 1	132 ± 1	134 ± 1
Arterial pressure (mm Hg)	47.9 ± 2.2	42.6 ± 1.1	46.7 ± 1.4
Venous pressure (mm Hg)	4.0 ± 0.6	3.2 ± 0.4	3.3 ± 0.5
Heart rate (beats/min)	167 ± 6	165 ± 7	165 ± 4
pH	7.34 ± 0.01	7.33 ± 0.01	7.33 ± 0.01
Pco ₂ (mm Hg)	54.5 ± 0.4	53.0 ± 0.9	53.9 ± 0.6
Po ₂ (mm Hg)	19.4 ± 1.3	19.4 ± 1.2	21.9 ± 1.4
Hematocrit (%)	33.7 ± 1.5	34.4 ± 1.0	33.3 ± 2.2
Protein (gm/dl)	3.7 ± 0.1	3.9 ± 0.1	3.7 ± 0.1
Urine flow (ml/min)	0.82 ± 0.17	1.06 ± 0.19	0.83 ± 0.17
Urine osmolality (mOsm/kg)	138 ± 11	144 ± 14	125 ± 7
Urine sodium (mEq/L)	38.3 ± 6.4	34.0 ± 6.5	34.2 ± 6.0
Urine potassium (mEq/L)	9.4 ± 3.2	12.8 ± 4.0	5.8 ± 2.2
Urine chloride (mEq/L)	29.1 ± 2.4	38.6 ± 6.9	27.6 ± 2.8
Plasma ANF (pg/ml)	165 ± 24	115 ± 20	165 ± 12
Plasma arginine vasopressin (pg/ml)	8.12 ± 2.12	4.03 ± 0.64	8.13 ± 5.20
Plasma angiotensin II (pg/ml)	32.5 ± 6.4	25.7 ± 5.4	30.3 ± 5.8

After the 30-minute control period, the ANF antiserum was injected into two groups of fetuses. The low-dose group received the antiserum at 1:2000 dilution, and the high-dose group received the antiserum at 1:400 dilution. The control group was given an injection of the diluent.

Low-dose group. In the low-dose group administration of the ANF antiserum lowered fetal plasma ANF concentration from control levels of 115 ± 20 pg/ml by 13 ± 7 pg/ml at 10 minutes (Fig. 1). However this decrease was not statistically significant (F = 1.19,

p = 0.33; two-way analysis of variance). Fetal arterial pressure decreased sharply by 1.6 ± 0.4 mm Hg (F = 2.02, p = 0.06), returned to basal levels by 10 minutes after this low-dose antiserum injection, and was unchanged for the remainder of the experiment. Fetal heart rate increased transiently by 10 ± 4 beats/min (F = 1.98, p = 0.07) and returned to basal levels within 10 minutes after the injection. Venous pressure did not change significantly at any time after the antiserum administration (F = 1.00, p = 0.42). There was a small increase in fetal blood volume of

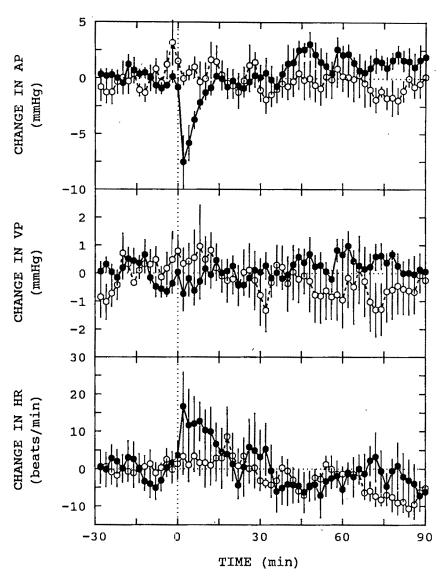


Fig. 2. Changes in fetal arterial pressure (AP), venous pressure (VP), and heart rate (HR) (mean \pm SE) after ANF antiserum injection at 30 minutes in high-dose (solid circles) and control (open circles) groups. See text for statistical analysis. Dotted line, Time of ANF antiserum injection.

 $1.8\% \pm 0.7\%$ at 10 minutes after the injection, but the change was not statistically significant (F = 1.73, p = 0.14). By 40 minutes, blood volume had returned toward baseline. Plasma protein concentrations did not change immediately after the low-dose antiserum injection but was elevated at 40 minutes after the injection (F = 3.81, p < 0.01). Thereafter, plasma protein remained elevated until the end of the experiment. Fetal arterial blood gases and pH did not change significantly throughout the experiment. Similarly, fetal urine flow rate; urine osmolality; and urinary sodium, potassium, and chloride excretion rates did not change in response to the low-dose antiserum treatment.

High-dose group. In the high-dose group injection of the ANF antiserum significantly reduced fetal

plasma ANF concentration (F = 6.17, p < 0.001) within 10 minutes by 65 \pm 14 pg/ml from control levels of 165 \pm 12 pg/ml (Fig. 1). Plasma ANF levels remained reduced for the duration of the experiment. Fetal arterial pressure showed significant changes after the high-dose antiserum injection (F = 4.22, p < 0.01; Fig. 2). There was an acute and transient fall in arterial pressure of 7.6 \pm 2.4 mm Hg from control, followed by a return to basal levels at 10 minutes after the injection. At 40 minutes, arterial pressure began to rise and reached a maximum increase of 3.0 \pm 1.1 mm Hg from control at 50 minutes after the injection (p < 0.05). Thereafter, arterial pressure was elevated for the remainder of the experiment. Venous pressure showed no consistent change throughout the experi-

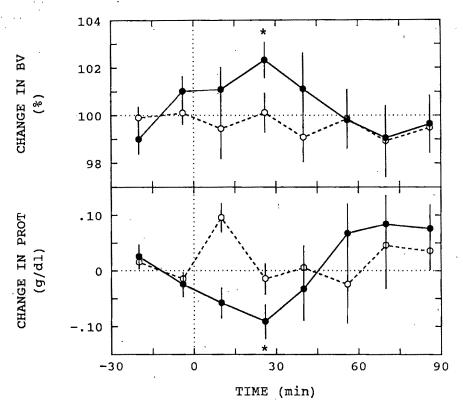


Fig. 3. Changes in fetal blood volume (BV) and protein (PROT) concentrations (mean \pm SE) after ANF antiserum injection at 30 minutes in high-dose (solid circles) and control (open circles) groups. Asterisk, p < 0.05 compared with control values during first 30 minutes. Dotted line, Time of ANF antiserum injection.

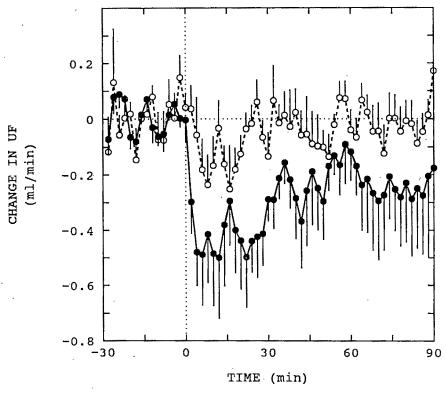


Fig. 4. Changes in fetal urine flow rate (UF, mean \pm SE) after ANF antiserum injection at 30 minutes in high-dose (solid circles) and control (open circles) groups. See text for statistical analysis. Dotted line, Time of ANF antiserum injection.

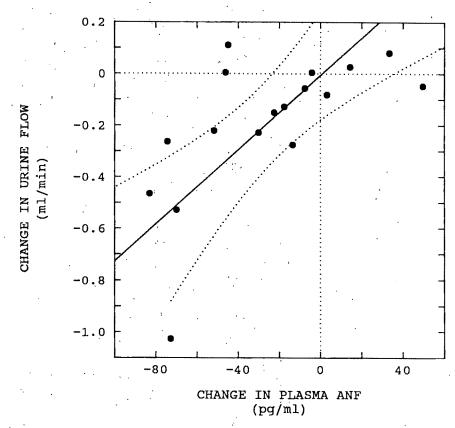


Fig. 5. Correlation between change in urine flow rate and change in plasma ANF concentrations in low-dose, high-dose, and control groups of fetuses during 30 minutes after ANF antiserum injection (r = 0.64, p < 0.01, n = 18).

ment (F = 1.05, p = 0.39; Fig. 2). The fetal heart rate changes were reciprocal to those in arterial pressure. Heart rate increased sharply by 17 ± 9 beats/min and gradually returned to basal levels by 20 minutes after the injection (F = 1.22, p = 0.30; Fig. 2). Heart rate continued to fall by a maximum of 7 ± 6 beats/min below basal levels at 50 minutes after the injection and slowly returned to control levels by the end of the experiment. Because of the variability among the animals, these changes in fetal heart rate were not statistically significant.

Fetal blood volume increased after the high-dose antiserum injection to a maximum of $2.3\% \pm 0.7\%$ above control at 25 minutes after the antiserum injection (F = 1.50, p = 0.22; Fig. 3) and returned to control levels thereafter. Concurrent with the increase in blood volume, there was a nonsignificant decrease from control in plasma protein concentrations at 25 minutes, which gradually rebounded to levels significantly above baseline at 50 minutes after the injection (F = 3.56, p < 0.01; Fig. 3). There were no consistent changes in fetal arterial blood gases and pH after ANF antiserum injection.

In response to the high-dose ANF antiserum, fetal urine flow rate was significantly suppressed within 5 minutes by 0.5 ± 0.1 ml/min from a basal rate of $0.8 \pm 0.2 \text{ ml/min}$ (F = 2.53, p < 0.05), and remained low at this level for 30 minutes. Thereafter, although urine flow increased somewhat, it remained below basal levels for the remainder of the experiment (Fig. 4). During the 30 minutes after antiserum administration, the reduction in urine flow was significantly correlated with the decrease in plasma ANF concentrations (r = 0.64, p < 0.01; Fig. 5). Urine osmolality increased by 33 ± 17 mOsm/kg from control at 25 minutes after the antiserum injection (F = 2.52, p < 0.05) but returned to baseline at 55 minutes. Urinary excretion rates of sodium, potassium, and chloride decreased significantly at 10 minutes after the injection $55\% \pm 12\%$ (Friedman's $\chi^2 = 15.2$, p < 0.01), $52\% \pm 10\%$ (Friedman's $\chi^2 = 17.0$, p < 0.01), and $54\% \pm 12\%$ (Friedman's $\chi^2 = 16.6$, p < 0.01) of control values, respectively. However, by 40 minutes after the antiserum injection, urinary electrolyte excretion rates were not different from basal levels (Fig. 6).

In fetuses of the control group, variations in cardiovascular variables during the entire experimental period were within the 95% confidence interval about the mean during the 30-minute control period. There were no significant changes in blood gases, pH, or renal function in these fetuses throughout the experimental period.

Hormone responses to ANF antiserum injection. In the low-dose and the high-dose groups, plasma angio-

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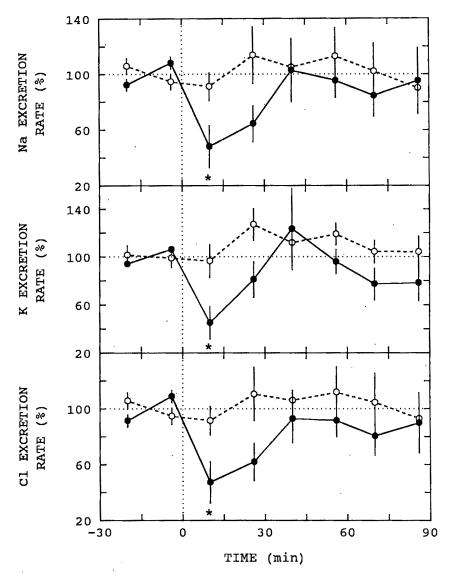


Fig. 6. Percent changes in sodium (Na), potassium (K), and chloride (Cl) excretion rates (mean \pm SE) after ANF antiserum injection at 30 minutes in high-dose (solid circles) and control (open circles) groups. Asterisk, p < 0.05 compared with control values during first 30 minutes of experiment. Dotted line, Time of ANF antiserum injection.

tensin II concentrations increased after the ANF antiserum injection (Fig. 7). The angiotensin II responses were similar in the two groups; therefore the data were combined for statistical analysis. At 10 minutes after ANF antiserum injection, angiotensin II concentrations were elevated by 22 ± 9 pg/ml from control levels of 28 ± 4 pg/ml (F = 2.55, p < 0.01). Angiotensin II concentrations returned toward basal levels by the end of the experiment.

In both the low-dose and high-dose groups, plasma arginine vasopressin concentrations during the 90-minute period after ANF antiserum administration were not significantly altered from the levels in the control period (Fig. 7).

Comment

To determine the role of endogenous ANF in the maintenance of fetal cardiovascular and renal functions, ovine fetuses were studied after suppression of endogenous ANF. Because of the unavailability of an effective antagonist for ANF, blockade was accomplished by immunoneutralization of endogenous ANF with a specific antiserum. Using similar techniques of immunoneutralization of ANF in adult rats, other investigators have reported that endogenous ANF is consistently involved in the maintenance of urinary output and renal sodium excretion. ^{13, 16, 17} The effect of ANF on arterial pressure was variable in that ANF antiserum treatment was shown to have either no effect. ¹³ or sig-

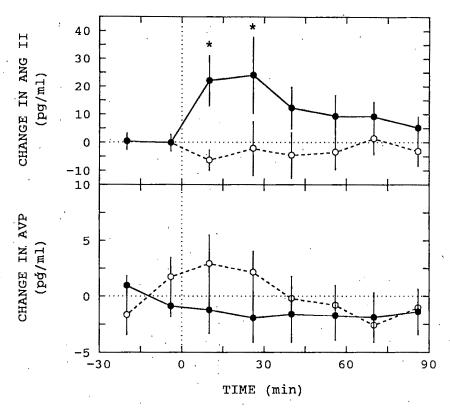


Fig. 7. Changes in plasma angiotensin II (ANG II) and arginine vasopressin (AVP) concentrations (mean \pm SE) after ANF antiserum injection at 30 minutes in control fetuses (open circles, n=5) and fetuses given both low-dose and high-dose-ANF antiserum (solid circles, n=13). Asterisk, p < 0.05 compared with control values during first 30 minutes. Dotted line, Time of ANF antiserum injection.

nificantly increased arterial pressure.¹⁷ In this study, when the antiserum was administered in sufficient quantities as a bolus to the ovine fetus, plasma ANF concentration was significantly and consistently reduced for at least 90 minutes. This reduction in circulating ANF levels resulted in significant alterations in fetal arterial pressure, blood volume, and renal excretion of fluid and electrolytes. These findings are consistent with the role of ANF as a physiologic regulator of vascular pressure and fluid balance in the fetus under basal conditions.

In the fetus, plasma ANF concentration is normally greater than that in the adult, 46 and the endogenous release rate of ANF is high at 13 ng/min/kg. The present study demonstrated that a high concentration of ANF antiserum was required to reduce plasma ANF to 58% of basal levels. The use of a lower dose (a fivefold higher dilution) of ANF antiserum reduced plasma concentrations to only 88% of basal levels. In this low-dose group, although ANF levels were below control levels after antiserum injection, at no time was this decrease significant. These observations are consistent with the high production rate of ANF in the fetus.

The initial arterial pressure response of the fetus to the high-dose antiserum injection was an abrupt but transient fall in arterial pressure. This fall in pressure was also seen in the low-dose group, although the decrease was much smaller in magnitude than in the highdose group. This decrease, however, was not detected in the control group of fetuses given 1% normal rabbit serum. This acute hypotension was an unexpected finding. It appeared to be related to the presence of the antiserum itself and might not be the result of the blockade of ANF action. Instead, it could be a nonspecific effect of the rabbit antiserum, such as the induction of complement activation by the antibody leading to histamine release, that caused a vasodilation and reduction in arterial pressure. The abrupt fall in arterial pressure would likely affect the fetal cardiovascular system. In studies where fetal hypotension was induced by nitroprusside, arterial pressure fell and remained reduced as long as nitroprusside was infused.18 In addition, all cardiovascular variables returned to normal within 10 minutes after termination of the nitroprusside infusion. In this study the arterial hypotension was transient; arterial pressure returned to baseline within 10 minutes and then increased to levels above basal conditions. This was contrary to a maintained hypotensive response such as that induced by nitroprusside. The transient nature in the fall in arterial pressure and the quick recovery suggested that it was not caused by the blockade of ANF action, because plasma ANF levels were reduced for a prolonged period. In previous studies of the adult rat^{18, 16, 17} a transient hypotensive effect after ANF antiserum administration was not reported. However, in those studies arterial pressure was measured at 15- to 30-minute intervals rather than the continuous recording and display (Fig. 2) used in this study. Thus it is unclear whether the transient fall in arterial pressure was missed by the previous investigators.

The reduction of plasma ANF concentrations to levels below normal in the fetus produced a delayed but sustained rise in fetal arterial pressure of $6.2\% \pm 2.2\%$ that was maintained for as long as plasma ANF concentration was suppressed. These findings are consistent with our previous report that infusion of exogenous ANF into the ovine fetus lowered arterial pressure.3 An increase in arterial pressure after ANF antiserum treatment has also been reported in the adult rat.17 The fetal heart rate responses to the ANF antiserum were reciprocal to those of arterial pressure, indicating a baroreceptor mechanism in the maintenance of fetal heart rate. A direct effect of ANF withdrawal on the fetal heart was not apparent. Fetal venous pressure was not consistently affected by the presence of the ANF antiserum. These results support the concept that, in the fetus, ANF normally participates in the maintenance of arterial pressure but not venous pressure or heart rate.

It has been demonstrated in the adult19 and the fetus3 that administration of ANF increases hematocrit, suggesting a reduction in blood volume. In the ovine fetus this decrease in blood volume is associated with a transient increase in plasma protein concentration. In this study blockade of endogenous ANF in the fetus resulted in a rise in blood volume (although not significant), accompanied by a fall in plasma protein concentration. These observations are consistent with a modulatory role of ANF in the transfer of proteins and fluids across the capillary in the fetus. However, urine flow rate was dramatically reduced at this time; therefore the retention of fluids by the fetal kidney might have contributed to the increase in blood volume. Although the acute, transient fall in arterial pressure might have allowed movement of fluid from the interstitium into the vascular compartment, causing an increase in blood volume, this appeared to be unlikely. This was because blood volume was not elevated until arterial pressure had returned to basal conditions. The elevation in blood volume was not maintained, even though plasma ANF concentrations remained suppressed. This might be partly due to the subsequent increase in arterial pressure in these fetuses.

The fetal renal response to ANF antiserum was much more pronounced than the cardiovascular effects. This was because of the observation that, after blockade of endogenous ANF, fetal urine flow rate was abruptly reduced to $41.4\% \pm 9.6\%$ of control values. The sharp, transient decrease in arterial pressure in response to ANF antiserum injection could have augmented the fall in urine flow rate, because it has been reported that in the ovine fetus hypotension induced by nitroprusside was accompanied by a fall in urine flow rate and urinary sodium excretion.20 However, in those studies the fall in urine flow was terminated as the hypotension was reversed. In the present study, the suppression of urine flow rate was prolonged even though the hypotension subsided. Thus it was unlikely that the long-term reduction in urine flow was the result of the short-term decrease in arterial pressure. Furthermore, plasma arginine vasopressin levels did not change during the entire experiment, thus eliminating its possible involvement in reducing urine flow. The fall in urine flow was most likely the result of the withdrawal of ANF, because a significant relationship existed between the fall in plasma ANF concentrations and the reduction in urine flow. This implies that by blocking the renal effects of ANF, urine flow was no longer maintained. Thus, under physiologic conditions, normal fetal urine flow appears to be maintained by circulating ANF levels. In support of this concept is the fact that plasma ANF concentration in the fetus is greater than in the adult, and urine flow rate is higher in the fetus than in the adult relative to body weight.

In conjunction with the suppression of urine flow, urinary excretion rates of sodium, potassium, and chloride were reduced. This reduction in electrolyte excretion might be the consequence of the blockade of the natriuretic action of ANF. However, the large concomitant reduction in fetal urine flow rate would have contributed to the decrease in urinary electrolyte excretion rates. The diuretic action of ANF is mediated through an increase in glomerular filtration rate at the fetal kidney.2 When ANF levels were reduced, a decrease in glomerular filtration rate would result. In this study, this was manifested as a fall in urine flow rate. With the reduction in glomerular filtration rate, the filtered load through the kidney was reduced, and thus the amount of electrolytes filtered decreased. The result was a fall in urinary excretion of the electrolytes. This fall in electrolyte excretion rate might be augmented by the action of aldosterone, because plasma aldosterone concentration was found to increase in response to ANF antibody injection in the rat. 13 However, this possibility is unlikely because of the observation in the ovine fetus that aldosterone stimulated the reabsorption of sodium but increased the excretion of potassium, leading to a decrease in the sodium-potassium ratio in the urine.21 This was inconsistent with our finding, where both sodium and potassium excretion rates

were reduced. Similarly in the studies by Sasaki et al.¹⁷ and Rudd et al.,¹⁸ the urinary excretion rates of both sodium and potassium were reduced after ANF antiserum treatment. Unlike urine flow that was continuously suppressed in parallel with the reduction in plasma ANF concentration, the excretion of electrolytes returned to basal levels. This increase in electrolyte excretion could not be due to a fall in plasma arginine vasopressin concentration, because arginine vasopressin levels did not change at any time after ANF antiserum treatment. This suggests that, although ANF may participate in the regulation of electrolyte excretion, factors other than ANF may contribute to the maintenance of urinary electrolyte excretion rate during normal conditions.

Reduction of endogenous ANF concentration in the fetus did not appear to affect plasma arginine vasopressin concentration. The lack of change in face of urinary changes further supports the role of ANF in the regulation of renal function in the ovine fetus. The reduction in circulating ANF levels increased plasma angiotensin II concentration. A similar increase in plasma renin activity in adult rats after ANF antiserum treatment has been reported by Naruse et al.16 The increase in angiotensin II concentration in the present study could have been due to the short-term fall in arterial pressure after ANF antiserum injection. Hypotension in the mature ovine fetus is a potent stimulus for increasing plasma renin activity.18 Although it is tempting to speculate that ANF is inhibitory to the renin-angiotensin system as seen in the adult,22 it is difficult to separate the effect of hypotension from that of ANF blockade in the stimulation of angiotensin II release. Besides, infusion of ANF into the ovine fetus was not associated with a fall in plasma renin activity.3

In summary, this study demonstrates that in the ovine fetus plasma ANF plays a role in the regulation of normal arterial pressure and blood volume and in the maintenance of basal urinary output. Furthermore, ANF appears to have little effect in the maintenance of fetal heart rate. Finally, an interaction of ANF with the other vasoactive hormones, angiotensin II and arginine vasopressin, appears to be minimal.

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A rabbit model for bacterially induced preterm pregnancy loss: Intervention studies with ampicillin-sulbactam

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We conducted experiments with a previously described rabbit model of *Escherichia coli*—induced preterm pregnancy loss. Does at 70% gestation were inoculated hysteroscopically with 0.2 ml of *Escherichia coli* (10^5 colony-forming units per milliliter) or saline solution. Animals were randomly assigned to either receive treatment with ampicillin-sulbactam (begun 1 to 2 hours before inoculation and continued for up to 7 days) or to receive no therapy. Animals were killed after delivery or after 7 days. Saline solution—inoculated animals had no pregnancy loss. Of the *Escherichia coli*—inoculated animals, those treated with ampicillin-sulbactam had significantly fewer deliveries, fewer positive cultures, and more live fetuses than the untreated animals ($p \le 0.001$). Cultures from multiple sites, amniotic fluid prostaglandin levels, and maternal progesterone levels were obtained, and the placenta, uterus, and fetal lung were histologically evaluated. In the second phase of the study, the *Escherichia coli*—inoculated animals were treated with ampicillin-sulbactam at one of three times: at inoculation or 2 or 4 hours after inoculation. The *Escherichia coli*—inoculated does treated with ampicillin-sulbactam at or before inoculation had significantly fewer deliveries, fewer positive cultures, and more live fetuses than the *Escherichia coli*—inoculated does in which treatment was delayed 4 hours ($p \le 0.01$). (AM J OBSTET GYNECOL 1991;165:1568-74.)

Key words: Intraamniotic infection, animal model, pregnancy loss

Clinically evident intraamniotic infection is a major cause of necnatal sepsis and maternal morbidity; subclinical infection has been implicated as a cause of preterm labor. The study of these processes in human beings is limited by ethical concerns, patient availability, and confounding variables. Therefore animal models are desirable for testing hypotheses on both clinical and subclinical infection in pregnancy.

Dombroski et al. developed a rabbit model that mimicked the presumed ascending pathway for the study of bacteria-induced preterm pregnancy loss. In this model, hysteroscopic inoculation of the lower uterine horns of pregnant does at 70% gestation was performed with bacterial inocula. In utero inoculation of Escherichia coli and Fusobacterium necrophorum produced prompt and reproducible pregnancy loss as well as clinical, microbiologic, and histologic evidence of infection. On the basis of this work we tested the null hypothesis that antibiotic treatment would not decrease pregnancy loss in this rabbit model when uterine horns were inoculated with E. coli.

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Material and methods

This protocol was approved by the Animal Use and Care Committee of the University of Colorado Health Sciences Center. Timed-pregnant New Zealand White rabbits (4.0 to 5.5 kg) were purchased from a vendor (Myrtle's Rabbitry, Thompson Station, Tenn.) and flown to Denver on day 14 or 15 of a 31- to 33-day gestation. The animals were housed in the Animal Resource Center of the University of Colorado Health Sciences Center. The animals were fed antibiotic-free pellets and hay and provided free access to water. The circadian cycle was 12-hour-day-12-hour-night lighting. On day 21 or 22, rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (5 mg/kg). After perineal and lower vaginal preparation with povidone-iodine solution, a hysteroscope (Storz 27020B, provided by Karl Storz Endoscopy-America Inc., Culver City, Calif.) was introduced vaginally and advanced into each cervix of the uterus didelphys. Once the hysteroscope was in the cervix, a sterile polyethylene cannula (0.06 inch outer diameter) was advanced through the operating port into the uterine horn for a distance of 6 cm. Through the tubing, 0.2 ml inoculum or pyrogen-free saline solution was injected with a tuberculin syringe.

E. coli ATCC 12014 was aerobically inoculated into thioglycollate broth and grown for 8 hours at 36° C

before concentration and washing. After centrifugation, the pellet was resuspended in sterile phosphate buffer and recentrifuged three times. A final suspension was then performed to achieve a final concentration of approximately 10⁵ colony-forming units per milliliter. Final concentrations were plated quantitatively before inoculation to confirm colony count. An aliquot remaining in each syringe was plated after inoculation to confirm viability.

Animals were randomly assigned to (1) inoculation with *E. coli* or saline solution and to (2) treatment with no antibiotic or ampicillin-sulbactam 100 mg/kg/day intramuscularly in three to four divided doses beginning 1 to 2 hours before bacterial inoculation and continued up to 7 days. After this series, 20 animals inoculated with *E. coli* were treated with ampicillin-sulbactam immediately after inoculation (0 hour), 2 hours after inoculation, or 4 hours after inoculation. In the second series, dosage was increased to 150 mg/kg/day when serum ampicillin-sulbactam levels in the initial animals showed values in the low therapeutic range.

After inoculation, animals were observed for signs of sepsis, delivery, or death. Animals were killed after delivery or after 7 days. Rectal temperatures were taken at surgery, at 24 hours after surgery, and at death.

At death, cultures were taken of blood, peritoneal fluid, amniotic fluid, and decidua and the cultures were inoculated onto 5% sheep blood agar, onto Mac-Conkey's agar, and into thioglycollate broth. Gram stains of peritoneal fluid, amniotic fluid, and decidua were prepared. Viability of remaining fetuses was noted. A blood sample for progesterone was obtained for determination by radioimmunoassay (Coat-a-Count progesterone iodine 125-labeled radioimmunoassay, Diagnostics Products Corp., Los Angeles). Pooled amniotic fluid from multiple sacs of individual does was collected for measurement of prostaglandin E2 and prostaglandin F2 metabolite by radioimmunoassay (M.J. Harper, San Antonio). These were the same prostaglandins measured in the rabbit model of Dombroski et al.1 The radioimmunoassay procedure was described by Harper et al.2 and modified by Jones et al.3 Samples were not extracted and indomethacin (10 μg/ml was added per tube). Lower uterine horn, placenta, and fetal lung sections were fixed in 10% neutral buffered formalin for histologic examination.

Outcomes were defined as follows: delivery, any fetus born before 7 days; positive culture, any organism grown in thioglycollate broth or on a plate, except for organisms grown outside the streaked area of the plate; live fetus present, any moving or breathing fetus delivered or remaining in utero; fever, temperature >104° F 24 hours after hysteroscopy. A rectal temperature of 104° F is 2 SDs above the mean in normal rabbits.

Histologic evaluation was based on the work of Mossman⁴ and Davies.⁵ A pathologic grading system of increasing severity from 0 to 4 was created by one of (R.H.S.) after one review of the slides for each of the following five items. Endometrial epithelium and glands: 0, no change; 1, rare or scattered degenerative changes with neutrophils or fibrin in glands; 2, diffuse degenerative changes with neutrophils or fibrin in glands; 3, same as grade 2 with superimposed focal epithelial or glandular necrosis; and 4, same as grade 2 with superimposed diffuse epithelial or glandular necrosis. Placental villus vessels: 0, unremarkable; 1, focal vasodilatation; 2, diffuse vasodilatation, 3, diffuse vasodilatation with focal disruption of vessels; and 4, diffuse vasodilatation and disruption of vessels. Neutrophilic infiltrate of membranes: 0, none; 1, rare sites; 2, occasional; 3, frequent; and 4, diffuse involvement. Necrosis of membranes: 0, none; 1, rare; 2, occasional; 3, frequent; and 4, diffuse involvement. Endometrial stromal edema: 0, none; 1, mild and focal or diffuse; 2, moderate and focal; 3, moderate and diffuse; and 4, marked and diffuse.

Data were entered into the SAS program (Statistical Analysis Systems, Inc., Cary, N.C.). Categoric data were analyzed by Fisher's exact test. Continuous data were analyzed by two-way analysis of variance and Student—Newman-Keuls test of multiple comparisons. Where assumptions were not met, nonparametric analysis consisted of the Kruskal-Wallis procedure. A *p* value of <0.05 was considered to be significant.

Results

Fifty animals underwent hysteroscopy. There were no uterine perforations. One maternal death occurred in an untreated animal 20 hours after inoculation with $E.\ coli$. Of nine rabbits receiving saline inoculum, four were untreated and five received ampicillin-sulbactam. There were no differences in outcome between treated and untreated saline solution—inoculated animals; therefore the two groups were analyzed together. When $E.\ coli$ —inoculated groups were compared, animals treated with ampicillin-sulbactam had fewer deliveries, fewer positive cultures, and more live fetuses than untreated animals (Table I). The final inoculum count varied from 2×10^4 to 6×10^6 . No differences in outcome were apparent for inoculum size.

Of animals at 22 to 24 hours, those inoculated with $E.\ coli$ and treated with ampicillin-sulbactam had fever less frequently than untreated animals. The only maternal death was excluded. None of nine saline solution–inoculated animals developed fever. Of $E.\ coli$ —inoculated animals, seven of nine (78%) untreated animals developed fever and none of the 11 (0%) treated animals developed fever (p < 0.001).

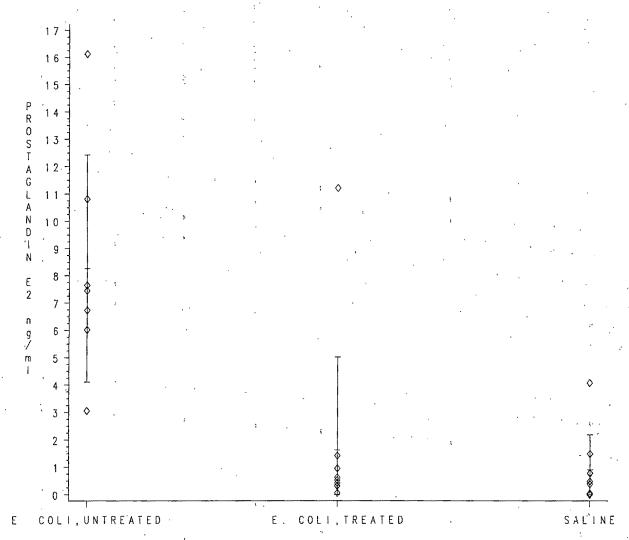


Fig. 1. Amniotic fluid prostaglandin E_2 (mean \pm SD) as related to intrauterine inoculum and treatment. Values for *E. coli*—untreated group are significantly higher compared with *E. coli*—treated group (p < 0.05) by Kruskal-Wallis procedure.

Table I. Outcome by intrauterine inoculum and treatment

Outcome	Inoculum								
	Saline solution			E. coli					
	Untreated ·		Treated		Untreated			Treated	
	No.	. %	No.	%	No.	%	<i>p</i> *	No.	%
Delivery Positive culture Live fetus present	0/4 0/4 4/4	. 0 0 100	0/5 0/5 5/5	0 0 100	10/10 10/10 0/10	100 100 0	0.001 0.001 <0.001	3/11 3/11 11/11	27 27 100

^{*}Fisher's exact test.

Prostaglandin E_2 and prostaglandin F_2 metabolite levels were higher in untreated E. coli—inoculated animals than in those treated with ampicillin-sulbactam (Figs. 1 and 2). Hemolysis was noted in five of seven amniotic fluid samples in untreated E. coli—inoculated

animals; in this group no differences were seen when hemolyzed and nonhemolyzed amniotic fluid prostaglandin levels were compared.

Progesterone levels were significantly lower in untreated E. coli—inoculated does than in saline solution—

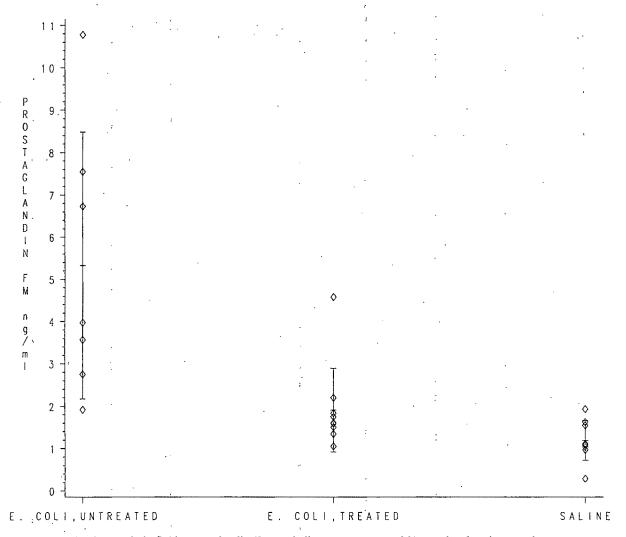


Fig. 2. Amniotic fluid prostaglandin F_2 metabolite (FM) (mean \pm SD) as related to intrauterine inoculum and treatment. Values for E. coli—untreated group are significantly higher compared with E. coli—treated group (p < 0.05) by Kruskal-Wallis procedure.

Table II. Positive cultures by site and treatment for E. coli inoculum

	E. coli inoculum					
	Untre	ated	Treated			
Site	No.	%	. No.	%		
Blood	9/10	. 90	0/11	0		
Peritoneum	8/10	80	1/11	9 '		
Amniotic fluid	9/10	90	0/11	0		
Decidua	10/10	100	2/11	18 . '		

inoculated controls. No significant difference was seen between progesterone levels of treated and untreated *E. coli*—inoculated rabbits, although the mean value of the untreated group was lower (Fig. 3).

Of animals inoculated with E. coli and not receiving ampicillin-sulbactam, cultures from all animals grew

this bacterium from multiple sites as the only organism. Furthermore, 9 of 10 animals had positive blood cultures (Table II). Three treated $E.\ coli$ —inoculated rabbits had positive cultures; in each of these, the culture from only one site was positive. Two of these grew $E.\ coli$ and one grew α -streptococci.

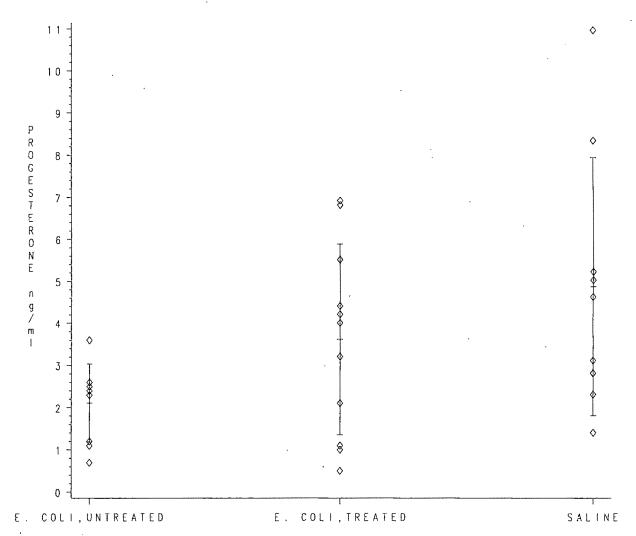


Fig. 3. Maternal serum progesterone (mean \pm SD) as related to intrauterine inoculum in treatment. Values for *E. coli*—untreated group are significantly lower compared with saline solution group (p < 0.05) by Kruskal-Wallis procedure. *E. coli*—untreated group was not significantly different from *E. coli*—treated group.

Table III. Pathologic grading by intrauterine inoculum and treatment

	Inoculum					
		E. coli				
Item	Saline solution (n = 9) (% grade > 0)	Untreated $(n = 9)$ (% grade > 0)	þ	Treated $(n = 11)$ (% grade > 0)		
Epithelium and glands	0	100	< 0.001	18		
Placental villus vessels	28	100	< 0.001	9		
Membrane necrosis	0	67	0.02	9		
Membrane neutrophils	0 .	44	0.12	9		
Endometrial stromal edema	23	100	NS	73		

NS, Not significant.

Results of the pathologic grading of the five parameters for each intrauterine inoculum are shown in Table III. In general, *E. coli*—inoculated animals that did not receive ampicillin-sulbactam had more severe changes

than either saline solution— or *E. coli*—inoculated animals treated with ampicillin-sulbactam. Untreated *E. coli*—inoculated animals were often grade 3 or 4 in multiple items. Treated *E. coli*—inoculated animals that

Oulcome	Hours from E. coli inoculum to treatment							
	1 to -2		0		2		4	
	No.	%	No.	%	No.	%	No.	%
Delivery	3/11	27*	0/4	0*	4/6	67	8/10	80*
Positive culture	3/11	27*	0/4	0*	1/6	17	6/10	60*
Live fetus present	11/11	100†	· · 4/4	100†	2/6	33	2/10	20†

Table IV. Outcome by E. coli inoculum and time of treatment

did not deliver did not have any grades higher than I. Saline solution-inoculated animals had only rare histologic changes. Two other findings were of note. First, there were no pathologic signs of infection in the lung fields; second, eosinophils were seen in the endometria of all untreated E. coli-inoculated animals, whereas they were not seen in any saline solution-inoculated animals.

In the second phase of the study, 4 animals were treated with ampicillin-sulbactam at inoculation with E. coli, 6 at 2 hours after inoculation, and 10 at 4 hours after inoculation. Animals inoculated with E. coli and treated at or before surgery had fewer deliveries, fewer positive cultures, and more live fetuses present than those treated 4 hours after inoculation (Table IV). Undelivered animals in the 2- and 4-hour groups had both live and dead fetuses in utero at death. In these cases, live fetuses were more often present in the distal uterine horns.

Comment

These experiments were designed to extend observations in the rabbit model developed by Dombrowski et al.1 to test whether antibiotic therapy would result in greater fetal salvage than no therapy. Through the use of saline solution controls and treated and untreated E. coli-inoculated groups, we confirmed that (1) the hysteroscopy itself did not result in pregnancy loss and (2) E. coli inoculation of pregnant uterine horns resulted in reproducible pregnancy loss. Our new observations are that treatment with ampicillin-sulbactam at or before inoculation resulted in improved outcome and that treatment 4 hours after inoculation led to worse outcomes than preinoculation treatment. The last finding is consistent with the classic work of Burke⁶ in which penicillin treatment of induced staphylococcal dermal lesions was ineffective after 4 hours.

Previous animal models of pregnancy loss have included intraperitoneal endotoxin,7 intravenous endotoxin,8.9 intraamniotic bacterial inoculation,10-12 and intracervical bacterial inoculation.13 Our premise was that hysteroscopic inoculation would closely mimic the presumed ascending pathway. Support for ascending in-

fection occurred in our experiments in which the antibiotic was delayed 2 or 4 hours after inoculation. If live fetuses were found after 7 days, they were present in the distal horns.

Comparison of treated and untreated groups revealed a model of overwhelming infection. By every measure of infection, including outcome, cultures, fever, and prostaglandin levels, we observed amelioration by administration of ampicillin-sulbactam before inoculation.

Prostaglandin levels were higher in untreated than in treated animals. We speculate that this could be caused by production of phospholipase A2 by E. coli,14 activation of amnion cells by endotoxin,15 or endotoxin activation of decidual macrophages to produce cytokines. Progesterone levels, which were not different in treated and untreated rabbits, were lower in untreated animals compared with saline solution controls. It is difficult to separate cause from effect in this situation. The numbers of animals were small, and the SDs were large.

Histologic findings, though variable in range in each of the five categories, confirmed the presence of significant inflammation and necrosis in the endometria and membranes of most untreated cases. Sections of treated undelivered animals were generally free of significant inflammation and necrosis in the endometria and membranes. Thus histologic evaluation correlated well with clinical outcome.

In spite of these dramatic findings, we recognize the limitations of the model when applied to human beings. First, antibiotics are begun at or before inoculation of organisms in the model (usually not the case in clinical medicine). Second, anatomic and pharmacokinetic considerations may not be comparable. Third, the rabbit is notoriously progesterone dependent in its mechanism of labor.16 Therefore, whereas the eradication of E. coli with improved pregnancy outcome is encouraging, it is premature to recommend routine treatment of intraamniotic infection without delivery in human beings on the basis of this work. Nevertheless, improved outcomes are reported in pregnancy loss models in the rhesus monkey,17 and case reports exist of successful

^{*}p < 0.01 When treatment ≤ 0 hour compared with 4 hours.

 $[\]dagger p$ < 0.001 When treatment ≤0 hour compared with 4 hours.

treatment of intraamniotic colonization¹⁸ and infection without delivery in human beings.¹⁹ Although the standard approach to intraamniotic infection is antibiotic treatment and delivery, our data imply that antibiotic treatment alone may suffice provided it is begun very early, before clinical manifestations of infection (i.e., in the subclinical period as manifested by preterm labor).

We have confirmed that hysteroscopic inoculation of *E. coli* into pregnant rabbits results in reproducible pregnancy loss accompanied by microbiologic and histologic evidence of infection. Furthermore, treatment with ampicillin-sulbactam at or before inoculation results in improved pregnancy salvage when compared with untreated animals or animals treated 4 hours after inoculation.

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LETTERS TO THE EDITORS

Hypertriglyceridemia in small-for-gestational-age fetuses

To the Editors: I read with great interest the article by Economides et al. (Economides DL, Crook D, Nicolaides KH. Hypertriglyceridemia and hypoxemia in small-for-gestational-age fetuses. Am J Obstet Gynecol. 1990;162:382-6). The authors found elevated plasma triglyceride concentration in samples obtained by cordocentesis for small-for-gestational-age fetuses.

One month earlier we described the increase of cord blood triglyceride concentration in small-for-gestational-age infants born to mothers with placental insufficiency. The mechanism of triglyceride increase in those cases is not well understood. I suggest that chronic fetal hypoxia caused by placental insufficiency is associated with a substantial release of catecholamines. Increased secretion of catecholamines leads to lipolysis and results in elevated blood fatty acid level. The fetal liver takes up some of the excess fatty acid and synthesizes endogenous triglycerides that appear in the fetal serum.

It has been found that the duration of fetal hypoxia must be considerable, perhaps even an hour or more, for it to result in hypertriglyceridemia.²

I proposed that the level of cord blood triglyceride may prove to be a useful indicator of chronic fetal hypoxia.

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Response declined

Splitting hairs about splitting muscles

To the Editors: Helmkamp and Krebs (Helmkamp BF, Krebs H-B. The Maylard incision in gynecologic cancer. Am J Obstet Gynecol 1990;163:1554-7) recently presented data substantiating the value of the Maylard abdominal incision. This article was timely, nicely done, and welcome. The Maylard technique, however, is not a "muscle-splitting" incision. It is, rather, a muscle-cutting procedure. Quoting Condon¹ in his chapter on appendicitis in Sabiston's Textbook of Surgery, "... the muscle-splitting incision (McArther-McBurney) is the time-honored approach and one widely used today. ... Its advantage is that separation of muscles in the line of their

fibers produces a wound that does not depend entirely upon sutures for restoration of tissue continuity." Surgical transection of the rectus muscles does not fit this description.

The risk of subfascial hematoma and the associated need for a subfascial drain are related to the creation of a closed space between the fascia and parietal peritoneum. Both can be eliminated by meticulous hemostasis and by allowing the parietal peritoneum to remain open and unsutured.²

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Reply

To the Editors: Thank you very much for your comments regarding our recent article. I agree that "musclecutting" more accurately describes the Maylard technique because the incision through the rectus muscles is perpendicular to the muscle fibers rather than parallel.

I do not suture the posterior rectus sheath in closing vertical and Pfannenstiel incisions. If you do likewise with the Maylard incision and have had no wound complications, it may be possible to eliminate the subfascial drain as you suggest.

B. Frederick Helmkamp, MD

3289 Woodburn Road, Suite 320, Annandale, VA 22003-6897

Effects of leukotrienes in the placental vasculature

To the Editors: Thorp et al. (Thorp JA, Walsh SW, Brath PC. Comparison of the vasoactive effects of leukotrienes with thromboxane mimic in the perfused human placenta. Am J Obstet Gynecol 1988;159:1376-80) have clearly shown that leukotrienes B₄ (LTB₄) and C₄ (LTC₄) do not exert major effects on the placental vasculature except at high doses (>1 μg per bolus injection). It is, however, clear from previous work that prostaglandins may have more potent effects on angiotensin II constricted placental vessels than when added alone¹; therefore we have investigated the effects of coadministration of LTB₄ or LTC₄ (5 to 500 ng per bolus injection) with angiotensin II (5 μg per bolus injection) on placental perfusion pressures. The perfusion system used in this study has been described.¹

Only the highest bolus injection (500 ng) of LTB₄ or LTC₄ increased the perfusion pressure (LTB₄, 17.8 ± 0.3 mm Hg; LTC₄, 6.2 ± 2.9 mm Hg; mean \pm SEM, n = 6); this finding agrees with the earlier study by Thorp et al. Angiotensin II (5 µg per bolus injection) stimulated a greater increase in perfusion pressure (34 \pm 8 mm Hg, n = 13); this was not affected by coadministration of 500 ng LTB4 or LTC4 $(36.7 \pm 13.3 \text{ and } 26.2 \pm 7.6 \text{ mm Hg}, n = 6, \text{ respec-}$ tively). These results support the contention of Thorp et al. that leukotrienes are not active modulators of the placental vasculature; it also demonstrates that the leukotrienes have no effect on contracted placental vessels. It is thus apparent that leukotrienes and prostaglandins differ markedly in their effects on the placental vasculature.

> M.H.F. Sullivan, PhD, P.R. Tranter, PhD, and M.G. Elder, MD

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REFERENCE

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Reply

To the Editors: We are pleased that Sullivan et al. have confirmed our results that leukotrienes are relatively weak vasoconstrictors in the human placental vasculature even when the vessels are first constricted with angiotensin II. It appears that most compounds other than thromboxane are relatively weak vasoconstrictors in the human placenta. This probably serves to protect the placenta from vasoconstriction and assure adequate perfusion for placental and fetal growth. As more information accrues, it appears that major vasoconstrictive disorders of the placenta such as preeclampsia involve an imbalance between thromboxane and prostacyclin. We believe that understanding the mechanisms regulating placental production of thromboxane and prostacyclin is extremely important and that research studies directed at uncovering these mechanisms will provide valuable information concerning the eventual treatment of various vasoconstrictive disorders of pregnáncy.

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james A. Thorp, MD, and Peter C. Brath, BS University of Texas Medical School at Houston, Department of Obstetrics, Gynecology and Reproductive Sciences, 6431 Fannin St., Suite 3.204, Houston, TX 77030

Infertility and eating disorders

To the Editors: We were very interested to see the results of the recent study of the eating behavior of women initially seen for infertility (Stewart DE, Robinson GE, Goldbloom DS, Wright C. Infertility and eating dis-

orders. AM J OBSTET GYNECOL 1990;163:1196-9). These results complement our findings. After administering the Bulimia Investigatory Test Edinburgh² (BITE) questionnaire to a large sample of women attending a gynecologic endocrinology clinic (n = 262), we found abnormal eating behavior in 24% of the population. Interestingly, the rate of abnormal eating behavior was significantly higher (p = 0.02) in women with ultrasonographic diagnoses of polycystic ovary syndrome (n = 153) than in a comparison group of women (n = 109) with other causes of menstrual disturbance. Thirty-one percent of women with polycystic ovary syndrome had abnormal BITE scores compared with 14% of women with other endocrinopathies. We would like to know if Stewart et al. analyzed their data according to diagnosis.

We strongly support the recommendation of Stewart et al. that the eating behavior of women attending fertility clinics or presenting with menstrual disorders be assessed. Abnormal behaviors such as binge eating or fasting should be rectified with appropriate therapy such as the program for bulimia described by Lacey.³ Pregnancies that occur in women with anorexia after ovulation resumes because of weight gain are more successful than pregnancies that occur when ovulation resumes because of pharmacologic stimulation.⁴ The 26-item Eating Attitudes Test⁵ is a suitable screening instrument. However, we found the BITE to be more sensitive and specific for bulimic behavior.

Sara McCluskey, BScHons, Chris Evans, BA, Hubert Lacey, MD, and Malcolm Pearce, MD

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Reply

To the Editors: It is indeed interesting that McCluskey et al. have recently found a prevalence similar to that reported by us of abnormal eating behavior in women attending a British gynecologic endocrinology clinic. However, although our women with abnormal menses scored significantly higher (p=0.0001) on the 26-item Eating Attitudes Test (EAT-26) than women with normal menses, after full investigation we found only 17% to have polycystic ovary syndrome.

McCluskey et al. state that the BITE is more sensitive and specific for bulimic behavior, which may account

for their slightly higher prevalence rates of abnormal eating behavior. Diagnostic criteria may also influence these rates: We adhered to the relatively strict criteria of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders. We also confirmed all possible cases identified by the screening instrument with a clinical interview, and we excluded false positives. The choice of screening instrument is clearly important. We note that other eating disorder investigators have expressed concern about the BITE,2 but we have no experience with that instrument. However, in this study we did compare the EAT-26 and another common eating disorder questionnaire, the Eating Disorder Inventory, which measures pathologic behavior across a broader range of psychologic and behavioral dimensions associated with eating disorders.3 Although these data were deleted for the sake of brevity in our article, we found the EAT-26 to be superior to the Eating Disorder Inventory in terms of sensitivity, specificity, brevity, and ease of scoring. The main source of error in both instruments, however, was denial of eating problems by some patients or false-positive results in overweight women who were unhappy, dieting, and preoccupied by food.

We again emphasize that body weight is often a poor predictor of eating disorders and that careful clinical inquiry is vital. Our earlier work indicates that women with eating disorders that remain unresolved before pregnancy have more problems during pregnancy, lower maternal weight gain, and smaller babies with lower Apgar scores than women whose eating disorders are in complete remission. The identification of eating disorders in reproductive endocrinology clinics is both cost-effective and an important opportunity for primary prevention.

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Indomethacin induces hypertensive crisis in preeclampsia irrespective of prior antihypertensive drug therapy

To the Editors: Schoenfeld et al. report preeclampsia being aggravated in two patients treated with β -adren-

ergic receptor blockers after ingesting indomethacin; the authors correlate this hypertensive crisis with antagonism of the β-adrenergic receptor blockers by indomethacin (Schoenfeld A, Freedman S, Hod M, Ovadia Y. Antagonism of antihypertensive drug therapy in pregnancy by indomethacin? Am J Obstet Gynecol 1989;161:1204-5).

I believe hypertensive crisis can occur in preeclampsia after ingestion of any prostaglandin synthetase inhibitor drug (aspirin, indomethacin) irrespective of the antihypertensive drug regimen, if any. Vasodilatory prostaglandins produced by the kidneys in the state of hypoperfusion maintain renal perfusion by dampening the vasoconstrictive effect of angiotensin on renal arteries. Indomethacin blocks prostaglandin synthesis and renders renal vasculature vulnerable to the constrictive effect of angiotensin II. Vasoconstriction of renal arteries results in renal hypoperfusion manifested by hypertensive crisis.

Animals given indomethacin and subjected to slight hemorrhage may have rapidly occurring severe renal damage.¹

Therefore it is wise to avoid all prostaglandin synthetase inhibitor drugs in women with pre-eclampsia.

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REFERENCE

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Reply

To the Editors: We thank Dr. Mousavy for his letter regarding our article. We agree with his statement about the interference with antihypertensive drugs by non-steroidal antiinflammatory drugs. In our article we stated that "indomethacin (and other) nonsteroidal antiinflammatory drugs vitiate the action of antihypertensive drugs and that in some patients the hypertensive response may be severe."

Dr. Mousavy's hypothesis, which deals with the antiprostaglandin synthesis mechanism, is based on the work of Vane¹ and likely explains a good portion of the action of the nonsteroidal antiinflammatory drugs. We would like to add that other, recently published^{2, 3} modes of action should be considered.

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 Zador, Hutzel Hospital, Department of Obstetrics and Gynecology, 4707 St. Antoine
 Blvd., Detroit, MI 48201. Fee: \$295 before

- 2/29/92, \$395 after 2/29/92. 25 CME credits and 25 Cognate credits. Tel.: (313) 745-7269. Fax: (313) 993-0689.
- Issues in Obstetrics and Gynecology, February 3-7, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.
- 4th Annual Review Course in Obstetrics & Gynaecology, May 6-9, 1992, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA Category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.
- Child Health 2000: World Congress and Exposition on Child Health, February 19-22, 1992, Vancouver, British Columbia, Canada. For more information contact: Child Health 2000, Suite 200, 1190 Melville St., Vancouver, British Columbia, Canada V6E 3W1. Tel.: (604) 684-3663. Fax: (604) 689-4806.
- 15th Annual Gold Coast OB/GYN Conference, "Current Concerns in High Risk Obstetrics and Gyn Oncology," February 10-12, 1992, Good Samaritan Medical Center, West Palm Beach, Florida. For information contact: Laura J. Keech, CME Coordinator. Tel.: (407) 650-6236.
- "Modern Concepts in the Management of Endometriosis," to air on Lifetime Medical TV. Important new information for primary care physicians and OB/GYNs on the diagnosis and treatment of endometriosis will be presented on Milestones In Medicine, a program which airs on Lifetime Medical Television and American Medical Television. Featuring guest host Robert L. Barbieri, MD, the program will examine the clinical basis for the emerging role of GnRH agonists and other modern therapies in the management of this often painful and debilitating disease. The program, made possible by an educational grant from Syntex Laboratories, Inc., is scheduled to air: every Sunday in December on Lifetime Medical TV at 6:30 PM ET/PT; on American Medical Television (on the Discovery Channel), on Dec. 15 and 22 at 10 AM ET; Dec. 13 and 26 at 2:30 PM ET on Héalth & Sciences Network.

OB GENERALISTS & SUBSPECIALISTS IN HIGH-RISK OBSTETRICS

18 5 /00

The Saudi Arabian Oil Company's (SAUDI ARAMCO) Medical Services Organization in Saudi Arabia needs an Obstetrician/Gynecologist with a subspecialty in maternal fetal medicine. American Board Certification and 2 years' experience after residency required. Strong leadership qualities as well as experience in both a professional and administrative capacity are preferred.

Saudi Aramco's 483 bed Dhahran Health Center has all major specialties and most subspecialties. The company also operates out-patient and emergency care clinics in its 3 surrounding communities. Saudi Aramco employees and their dependents comprise the 230,000 patient population. Our Blood Bank is accredited by the American Association of Blood Banks. Consulting liaisons exist with the WHO in Geneva and the Centers for Disease Control in Atlanta.

As a Saudi Aramco employee, you will receive a competitive base compensation package as well as an expatriate premium. Additional benefits include noncontributory group life insurance, company-matched savings, free medical care at Saudi Aramco's hospital and clinics, housing inside a company community, and extensive recreation facilities and activities. Your eligible children will be enrolled in company schools, comparable to U.S. private schools. Annually, there are up to 13 company holidays plus you will be eligible for 36 calendar days of vacation, with round-trip airfares to the U.S. or Canada for you and your family.

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GREG STELMACH JACKSON AND COKER, INC. 115 Perimeter Center Place Suite 380 11266 Atlanta, GA 30346 Tel. 1-800-544-1987



The Louisiana State University Medical Center in New Orleans is recruiting for a Chairman for the Department of Obstetrics and Gynecology. The individual must be Board Certified in OB/ GYN, possess a well established reputation as an investigator and teacher. Send curriculum vitae and three references to: Charles V. Sanders, MD, Professor and Chairman, Department of Medicine and Chairman OB/GYN Search Committee, LSU School of Medicine, 1542 Tulane Avenue, New Orleans, LA 70112, LSUMC-NO is an equal opportunity employer

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Maimonides Medical Center is a progressive 705-bed teaching institution located in the residential Boro Park section of Brooklyn, 15 minutes from Manhattan. We are currently seeking a Director of Maternal and Fetal Medicine to supervise all clinical and educational activities within the Division. The successful candidate will be Board Certified and will demonstrate prior experience in the management of high-risk pregnancies. Responsibilities will include Resident training and supervision in didactic and clinical areas. This full-time position offers a competitive salary and benefits program and the opportunity for an academic appointment. Qualified candidates are invited to submit CV's to:

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Assistant Vice President
Human Resources
Maimonides Medical Center
4802 Tenth Avenue
Brooklyn, NY 11219



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We offer a generous salary and benefits package which includes Professional Liability Insurance, vacation, health insurance coverage, dental and prescription plans, and paid conference time. Newark, Delaware is in close proximity to Philadelphia and Baltimore, sports, cultural events, theatre and shopping.

If you like living in a great area, a diversity of patients and responsibilities, and a promising future, then direct your inquiries and curriculum vitae to:

Medical Center of Delaware c/o Garrett H. C. Colmorgen, M.D. Obstetrics and Gynecology Maternal-Fetal Medicine 4755 Ogletown-Stanton Road P.O. Box 6001 Newark, DE 19718

FULL-TIME FACULTY POSITION GENERAL OB/GYN

Eastern Virginia Medical School of MCHR is seeking a full-time faculty member to expand an established generalist division. Ideal candidate will be Board certified or actively seeking certification. Responsibilities include resident and student teaching on busy clinical services as well as private patient care. Outstanding research facilities are available. The department is committed to the continued growth of a dynamic generalist division. Competitive salary and benefits; academic appointment commensurate with experience.

EVMS is located in beautiful Tidewater Virginia, a metropolitan area of 1.3 million, Located close to the Chesapeake Bay and Atlantic Ocean, the area offers year-round recreational activities, mild climate, and a great family environment.

For more information, call or send CV to:

Kathleen McIntyre-Seltman, M.D. Director, Benign Gynecology Hofheimer Hall-6th Floor 825 Fairfax Avenue Norfolk, VA 23507 (804) 446-7412

William E. Gibbons, Chairman Dept. OB/Gyn Hofheimer Hall-6th Floor 825 Fairfax Avenue Norfolk, VA 23507 (804) 446-8934

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The three community clinics are satellite facilities of an 1100 bed multilevel county institution located in a suburb 25 miles south of Chicago. The satellite clinics provide comprehensive primary health care services to residents in surrounding communities. Responsibilities include prenatal examinations, primary clinical care, plans for delivery and post partum care.

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1-800-468-3333 for informational packet.

FACULTY POSITION General Obstetrics & Gynecology

The Department of Obstetrics & Gynecology at The Ohio State University College of Medicine is seeking a board-certified or boardeligible physician in General Obstetrics & Gynecology for a faculty position at the rank of assistant professor. An excellent opportunity exists for research, teaching and clinical practice.

Send curriculum vitae and/or contact Steven Gabbe, M.D., Professor and Chairman, or Nina Smith, M.D., Assistant Professor and Acting Division Director, Department of Obstetrics and Gynecology, Ohio State University Hospitals, 505 Means Hall, 1654 Upham Drive, Columbus, Ohio 43210.

Telephone: (614) 293-8697

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FACULTY POSITION Gynecologic Oncology

The Department of Obstetrics & Gynecology at The Ohio State University College of Medicine is seeking a board-certified or board-eligible physician in Gynecologic Oncology for a faculty position at the rank of assistant professor. An excellent opportunity exists for research, teaching and clinical practice.

Send curriculum vitae and/or contact Steven Gabbe, M.D., Professor and Chairman, or Larry Copeland, M.D., Professor and Division Director, Department of Obstetrics and Gynecology, Ohio State University Hospitals, N500 Means Hall, 1654 Upham Drive, Columbus, Ohio 43210.
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For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE



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Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.



Obstetrics and Gynecology

Ravenswood Hospital Medical Center is currently recruiting full-time Staff Physicians in Obstetrics and Gynecology to serve a diverse and growing patient population in northwest Chicago. We have a newly renovated single room maternity unit, that does over 2,000 deliveries annually, and a Level II nursery. As a 350-bed teaching hospital affiliated with the University of Illinois, we also feature Internal Medicine, Family Practice and Transitional Residency Programs. Plus, our Wenske Laser Center provides educational and research opportunities in advanced techniques in laser gynecologic surgery. We are conveniently located in a pleasant urban neighborhood with easy access to the Loop, Lake Michigan and northern suburbs.

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Please forward C.V.'s in confidence to: Michael S. Baggish, M.D., Chairman of Obstetrics and Gynecology, Ravenswood Hospital Medical Center, 4550 North Winchester, Chicago, IL 60640-5205. Equal Opportunity Employer M/F.

Ravenswood Hospital Medical Center

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D. Chairman
Department of Obstetrics/
Gynecology
University of Wisconsin
Medical School
600 Highland Avenue,
H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D. Chairman and Chief Milwaukee Campus Department of Obstetrics/ Gynecology University of Wisconsin Medical School Sinai Samaritan Medical Center 2000 West Kilbourn Avenue Milwaukee, WI 53233

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FACULTY POSITION AVAILABLE

GENERALIST IN OBSTETRICS AND GYNECOLOGY

DEPARTMENT OF OBSTETRICS
AND GYNECOLOGY
UNIVERSITY OF MEDICINE AND DENTISTRY
OF NEW JERSEY
ROBERT WOOD JOHNSON MEDICAL SCHOOL

The Department of Obstetrics and Gynecology of the UMDNJ-Robert Wood Johnson Medical School invites applications for 4 full-time faculty positions as Generalists in Obstetrics and Gynecology. Candidates should be board eligible or certified in Obstetrics an Gynecology. The position involves academic practice, graduate and undergraduate medical education, and clinical and/or laboratory research. Academic rank and competitive salary commensurate with level of experience and qualifications.

Robert Wood Johnson Medical School is located in New Brunswick, New Jersey which is 30 minutes from New York City and Princeton and 45 minutes from Philadelphia. It is a college town surrounded by lovely suburbs. Art and cultural program flourish and we are in close proximity to seaside resorts. Please send curriculum vitae to: Robert Knuppel, M.D. Chairman, Obstetrics and Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019 The UMDNJ is an Affirmative Action/Equal Employment Opportunity Employer M/F/H/V and a member of the University Health System of New Jersey

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Innovative and ambitious professionals with board certification or eligibility are encouraged to apply. Please send c.v. or call Mary C. Hines, Physician Recruitment Manager, on 1-800-336-1442 or, in the D.C. Metro area, (202) 364-7442.

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Pamela J. Tropper, MD, Director, Maternal-Fetal Medicine, (201) 977-2499.



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A fellowship in Maternal-Fetal Medicine has been developed to emphasize basic and clinical research.

The first year of the Fellowship will be spent at the Robert Wood Johnson Pharmaceutical Research Institute in laboratory medicine. The second year will combine the clinical trials of the Pharmaceutical Research Institute and other protocols.

The Robert Wood Johnson Program offers the opportunity to collaborate with internationally renowned programs in microbiology, immunology, molecular genetics and pathology. Active epidemiologic research is ongoing. There are 7,300 deliveries in the Robert Wood Johnson Program. A Masters degree in Public Health may be incorporated. A Ph.D. background is preferable, but not a requisite.

Send inquiries and curriculum vitae to: Robert Knuppel, M.D., M.P.H., Professor and Chairman, Obstetrics and Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019. The UMDNJ is an Affirmative Action/Equal Opportunity Employer M/F/H/V and a member of the university Health System of New Jersey



DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

William C. Keettel, M.D. Professorship

The Department of Obstetrics and Gvnecology at The University of Iowa College of Medicine is seeking to fill the William C. Keettel M.D. Professorship. This endowed M.D. chair honors the late Dr. Keettel. A one to three year appointment is envisioned. Duties include clinical care and resident and medical student education. We are seeking an individual with a long record of distinguished scholarship, eligible for the academic rank of Professor, and Board certified in Obstetrics and Gynecology. Special expertise in an area of academic medicine is a desired qualification. Interested candidates should contact Jennifer R. Niebyl, M.D., Professor and Head, Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA 52242. The University of Iowa is an affirmative action equal opportunity employer. Women and minorities are encouraged to apply.



DIRECTOR MATERNAL FETAL MEDICINE

Department of Obstetrics & Gynecology University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

The Robert Wood Johnson Medical School invites applications for **Director of Maternal-Fetal Medicine**. The position of Director requires an individual with an established and broad understanding of medical education, research, patient care as well as effective administrative skills. Applicants should possess qualifications commensurate with those of a Professor of Obstetrics and Gynecology as defined by the Robert Wood Johnson Medical School.

The Division is located at St. Peter's Medical Center, the largest obstetrical and neonatal program in the State. Maternal-Fetal Medicine consists of five perinatologists, four sonographers, geneticist, two research nurses and one epidemiologist/biostatistician. The Robert Wood Johnson Program supports 7,000 deliveries per year.

Interested individuals should forward their curriculum vitae to: Robert A. Knuppel, M.D., M.P.H., Professor and Chairman, Department of Obstetrics & Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Piace, CN 19, New Brunswick, NJ 08903-0019.

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BRIGHAM AND WOMEN'S



Urogynecologist

The Department of Obstetrics, Gynecology, and Reproductive Biology at Brigham and Women's Hospital, a major teaching hospital of Harvard Medical School, seeks a full-time Urogynecologist to direct a clinical and educational program in urogynecology.

Applicants must be Board-certified in Obstetrics and Gynecology, and must have advanced training and experience in the medical and surgical aspects of urogynecology.

Academic rank at Harvard Medical School will be commensurate with experience, training, and achievements.

Strong interpersonal skills and commitment to clinical care, research, and teaching are essential.

Send inquiry and curriculum vitae to:

Kenneth J. Ryan, M.D. Chairman, Department of OB/GYN Brigham and Women's Hospital 75 Francis Street, ASB1-3-073 Boston, MA 02115

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DEPARTMENT CHAIR OBSTETRICS/GYNECOLOGY

GROUP HEALTH ASSOCIATION, a Washington D.C. area health maintenance organization with a membership of 155,000, seeks a chairperson to direct planning, organization and delivery of medical services for their busy Ob/Gyn Department. Group Health is a staff model HMO dedicated to excellence in primary and specialty medical care. Our 220 physicians spanning all medical specialties provide care to members in nine multispecialty medical centers and in superior community hospitals.

The successful candidate will be responsible for daily operations of the department, including supervision and evaluation of staff ob/gyns and certified nurse midwives.

Prospective candidates must be board certified with strong clinical background and three to five years post-residency administrative experience. This is a challenging opportunity for a professional who has demonstrated a high level of competency in clinical leadership and management and possesses excellent communicative and collaborative abilities. Direct patient care responsibilities required.

Interested candidates may send curriculum vitae and statement to:

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Medical Director
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FELLOWSHIP IN PELVIC SURGERY UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL

Department of Obstetrics and Gynecology University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

A comprehensive one-year training program in pelvic surgery is being offered by the Division of Pelvic Surgery in the Department of Obstetrics and Gynecology at UMDNJ-Robert Wood Johnson Medical School.

The program is designed to train fellows in all aspects of gynecological surgery including: Perioperative Care, Radical Surgery for Gynecologic Malignancies, Urodynamic Evaluation, Treatment of Incontinent Women, Vaginal Reconstructive Endoscopic Pelvic Surgery, Surgical Intensive Care Management, Management of Breast Disease.

The fellow will be actively involved in a number of clinical research projects and will receive instruction in the design of clinical investigations and data analysis. The position is ideally suited for individuals seeking a career in academic gynecology.

Interested candidates should send their curriculum vitae to: Nicholas Kadar, M.D., Director, Division of Pelvic Surgery, Department of Obstetrics & Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019.

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excellent opportunity for a BC/BE obstetrician. We're located in beautiful Southeastern New Hampshire, where you'll discover the ideal lifestyle. The location offers proximity to mountains, lakes and seacoast, plus a wealth of cultural attractions. It all adds up to a very healthy family environment.

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Generalist Obstetrician/Gynecologist Maternal/Fetal Medicine Subspecialist Gynecologic Oncologist Faculty Positions

The James H. Quillen College of Medicine of East Tennessee State University (ETSU) is seeking a board certified/eligible General Obstetrician/Gynecologist and a board certified/eligible Maternal/Fetal Medicine (Perinatology) subspecialist and a board certified/eligible Gynecologic Oncologist. These positions offer an excellent opportunity combining research, teaching and private practice with a faculty position at the Assistant/Associate Professor level. Salaries are very competitive and fringe benefits are excellent. The Medical School is located in the Tri-Cities area of Northeast Tennessee in a beautiful section of the Appalachians and within sight of the Blue Ridge Mountains. The area has excellent hunting, fishing, and camping with multiple ski resorts at your doorstep. The Tri-Cities combines good schools and rural living in a safe, beautiful environment. Yet, the medical referral base consists of 1.3 million people within a 50 mile radius of the Medical School. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman, Dept. OB/GYN
James H. Quillen College of Medicine
East Tennesee State University
P.O. Box 19570A
Johnson City, TN 37614-0002

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DIRECTOR OF MATERNAL AND FETAL MEDICINE

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The University of Illinois College of Medicine at Chicago invites applications and nominations for the position of Director of Maternal and Fetal Medicine in the Department of Obstetrics and Gynecology. Academic rank dependent upon qualifications. Candidates should be certified in Maternal/Fetal Medicine, have substantial scholarly productivity in the field, and have ability to provide dynamic leadership in administering a comprehensive program of patient care, education, and research in Obstetrics. Interested individuals should send a curriculum vitae to:

Antonio Scommegna, M.D.
Professor and Head
Department of Obstetrics and Gynecology
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Department of Obstetrics and Gynecology University of Iowa College of Medicine

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Send curriculum vitae to:

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Professor and Chairman, Department of OB/GYN
UNIVERSITY OF TENNESSEE MEDICAL CENTER at
KNOXVILLE, 1924 Alcoa Highway
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Search Committee c/o Millard Simmons, M.D. Chief, Division of Maternal-Fetal Medicine Department of Obstetrics and Gynecology West Virginia University, School of Medicine Health Sciences Center Morgantown, WV 26506

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Salary and academic rank commensurate with curriculum vitae and experience

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 Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. J Reprod Med 1989;34(8):540-542.

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December 1991 in two parts, part 1 volume 165, number 6 Index number

American Journal

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PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve sportaneously or may require drug discontinuation or surgery.
Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should not use SYNAREL
if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the
dose. Coursel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL
if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are altergite to any of its ingredients. 3. Sale
use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during
treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician
A Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation
occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6
month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should
not be important. In patients with major risk factors for foecreased bone mineral control, i.e., chronic alcohol and/or
tobacco use,

gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 550 times the maximum recommended human irransal dose respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in Harderian gland tumors in males and an increase in the relative times and the relative time of the relative times and the relative time of the relative times and the relative times and time of the relative bioavailability to sentility of rettility of rettility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: Pregnancy Category X. Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 tetuses at the hipsels dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 µg/kg/day and 018 µg/kg/day, respectively, failed to demonstrate an increase in fetal mortality and a decrease in fetal wortality and a decrease in fetal wortalities.

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Nursing Mothers: Itis not known whether SYNAPILL is excreted in human milk, Because many urups are excreted in human milk, and because the effects of SYNAPIL or lactation and/or the breasted child have not been eletermined, SYNAPIL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAPIL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenile: hot flashes (SYNAPIL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insominia (8%, 49%), Androgenic: area (13%, 20%), raylogia (10%, 23%), effective breast size (10%), 69%), ebema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%), Local: nasal irritation (10%, 3%); Miscellaneous: depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthrafigal, in clinical trials, immediate hypersensitivity thought to be possibly or probably related to naferelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total wortebral bone mass, measured by quantitative computed tomography (OCT), decreased by an awerage of 87% and 43%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment, the average trabecular bone dernsity and total bone mass were 4.9% and 3.3% less than pretreatment levels. Breatment Mean total vertebral amass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decreased in the mineral content recommended or monits of impresence of other instantiana, and the first total bone loss.

Changes in Laboratory Values: Plasma enzymes. During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids: At enrollment and at end of treatment, 9% of patients on SYNAREL 400 µg/day and 2% on danazol had total

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 µg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL. Mean (±SEM) pretreatment values for total cholesterol from all patients were 1918 (4.3) mg/dL in the SYNAREL group and 1931 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 2077 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant (p < 0.05) in both groups.

Triglycer/des were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) com-

patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 90 mg/dL) compared with 15% of those on danazol. There was no increase in the LDLHDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDLHDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with elevations of plasma of the properties of the matocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.





CURRENT PROBLEMS IN OBSTETRICS, GYNECOLOGY AND FERTILITY

Editor-in-Chief: Robert L. Barbieri, MD State University of New York at Stony Brook

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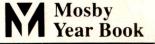
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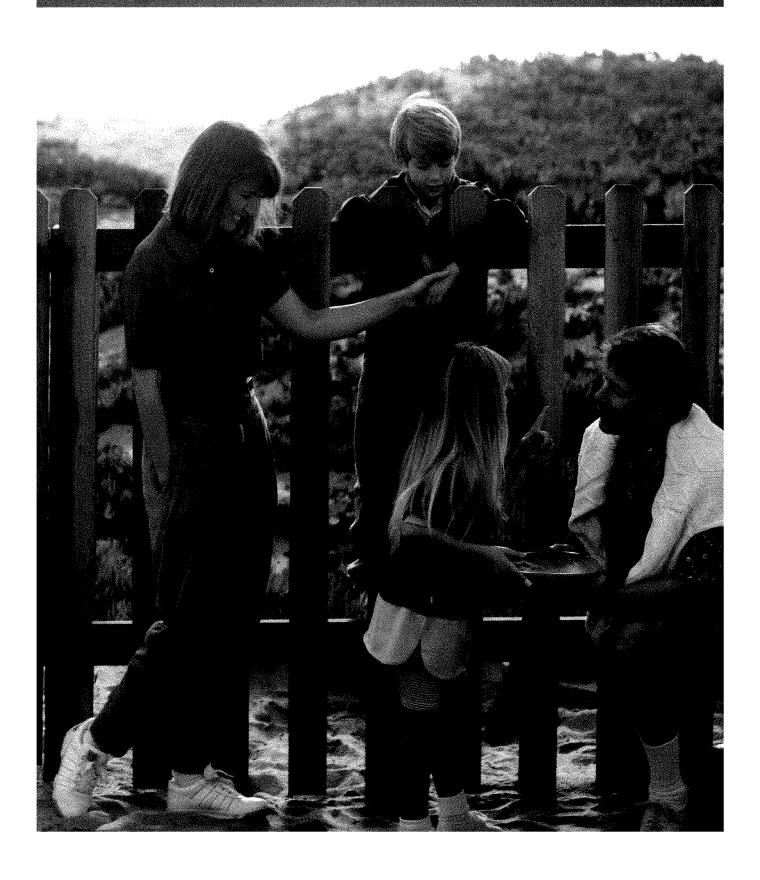
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NORPLANT SYSTEM levonorgestrel implants

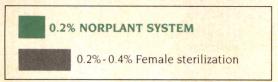


Six reasons why you can feel comfortable recommending it to your patients

Effectiveness comparable to tubal ligation

Although no contraceptive is 100% effective, the NORPLANT SYSTEM is one of the most effective forms of birth control ever developed. The average annual gross pregnancy rate over a five-year period is less than 1%.

Lowest expected and typical failure rates with NORPLANT SYSTEM vs. sterilization during first-year use



Adapted from Trussell I, et al.

Long-term convenience... estrogen-free

NORPLANT SYSTEM, a progestin-only contraceptive, is an excellent option for women desiring a long-term method. Its effectiveness lasts for five years and is not dependent on compliance.

Reversible, with prompt return to previous level of fertility

NORPLANT SYSTEM is reversible at any time; the contraceptive effect ceases when the capsules are removed.

In one study, 40% of women wanting to conceive did so within 3 months of removal, 63% by 6 months, 76% by 12 months, and 90% by 24 months.²

High patient acceptance despite menstrual pattern changes

NORPLANT SYSTEM causes menstrual irregularities (the most common complaint),* which occur in many women during first-year use and diminish over time. Only 9.1% of patients discontinued during first-year use due to these changes.^{3†}

Women who are thoroughly informed of potential menstrual changes and side effects are more tolerant of them when they occur.⁴

Well-received, well-liked by American women

One study of 140 users revealed that 94% were satisfied with NORPLANT SYSTEM, 91% recommended it to their friends, and 74% wanted to use it again.⁵

A 10- to 15-minute office procedure

A local anesthetic and a small 2 mm incision...little or no discomfort during insertion...proper placement should not leave a noticeable scar...in most cases sutures are not needed.

- *Serious as well as minor side effects may occur (see prescribing information).
- † Based on multicenter trials of 2,470 women.

Please see brief summary of prescribing information on adjacent page.



Lasts 5 years...yet is reversible



BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage
The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

Active throughpublishing at throughpublic disorders. 2. Undiagnosed absormal position.

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver turnors. 5. Known or suspected carcinoma of the breast.

tumors. 5. Known or suspected carcinoma or the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. Bleeding Irregularities — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the cansules must be removed. whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. Delayed Follicular Atresia — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles that are clinically indistinguishable from varian cysts.

follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be

required.

3. Ectopic Pregnancies — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out extonic pregnancy in any patient pregenting with lower-abdominal pain.

ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. Breast-feeding — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after

Foreign-body Carcinogenesis — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to

6. Thromboembolic Disorders — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. Cigarette Smoking — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in yourne cure 25 vocased.

quite marked in women over 35 years old.

2. Elevated Blood Pressure — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. Thromboembolic Disorders and Other Vascular Problems — An increased risk of

thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

relative risk is 4- to 11-foid injoher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is

Induction (MI). An increased risk of MI has been attributed to combined of use. Insist thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levynorgestic-containing OCs.

levonorgestrel-containing OCs.

4. Carcinoma — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. Hepatic Tumors — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal

hemorrhage.

6. Ocular Lesions — Retinal thrombosis is associated with OC use and is believed to be related to 6. Ocular Lesions — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately. 7. Use Before or During Early Pragnancy — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. Gallbladder Disease — Early studies reported an increased lifetime relative risk of gallblade surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

Precautions
GENERAL

1. Physical Examination and Follow-up — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and least annually during its use. Exams should include special reference to the implant site, bloo pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant labor tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules silve monitored with particular care.

be monitored with particular care.

2. Carbohydrate Metabolism — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appea minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYS Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Sc progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See

Warnings.)

3. Liver Function — Consider removing capsules if jaundice develops. Steroid hormones may poorly metabolized in patients with impaired liver function.

4. Fluid Retention — Steroid contraceptives may cause some degree of fluid retention. Presc

with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid

Emotional Disorders — Consider removing capsules if significant depression occurs since symptom may be drug-related. Observe carefully those with history of depression and consic

removal if depression recurs to a serious degree.

6. Contact Lenses — Contact-lens wearers who develop visual changes or changes in lens

tolerance should be assessed by an ophthalmologist.

7. Insertion and Removal — Insertion is advised during the first 7 days of the cycle or immer following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-fi women. Follow insertion and removal instructions closely. Healthcare professionals are stror advised to be instructed in the procedures before they attempt them. Proper insertion just un skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. capsules cannot be removed a first attempt, attempt, removal attempt when the site has headed.

Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occu implant site but is usually reversible following removal. See Full Prescribing Information for Detailed Insertion/Removal Instructions.

8. Infections — Implant site infection has been uncommon (0.7%); aseptic technique and pre insertion/removal reduces possibility of infection. Institute treatment if infection occurs; removables it infection process.

capsules if infection persists.

capsules if infection persists.

9. Expulsion — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replace Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. Provisions for Removal — Advise women that capsules may be removed at any time for reason. Personnel instructed in removal technique should perform removal on request or at to of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disea Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has beer reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYS users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptak

increased.

CARCINOGENESIS: See Warnings section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See Warnings section and Full Prescribing Information.

NURSING MOTHERS: See Warnings section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to try patient. Advise patients that Prescribing Information is available upon request. Inform prosper users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed contraceptions, and benefits associated with insertion/removal procedures. from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions
The following have been associated with the NORPLANT SYSTEM during first year of use: ma bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregulz (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain o itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).
Controlled clinical studies suggest that the following, occurring during the first year, are prob associated with NORPLANT SYSTEM use; headache; nervousness; nausea; disziness; adnexa enlargement; dermatitis; acne; change of appetite; mastalgia; weight qain; hirsutism, hypertri and scalp-hair loss. The following were reported with a frequency of 5% or greater during the year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis. musculoskeletal pain; abdominal discomfort; leukorrhea; vaoinitis,

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregula Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic* capsules, each containing 36 mg of the pro levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six cap should be performed during the first 7 days of the onset of menses by a healthcare profession instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midp of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanilike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. (See Full Prescribing Information for Detailed Insertion/Removal Instructions.) CI 4064-1 12/10/90

References: 1. Trussell J, Hatcher RA, Cates W, et al: Stud Fam Plann 1990;21:51-55. 2. Sivin I, Diaz S, Holma P, et al: Stud Fam Plann 1983;14:1.
191. 3. Data on file, Wyeth-Ayerst Laboratories. 4. Alvarez-Sanchez F, Brac V, Faundes A: Stud Fam Plann 1988;19:118-121. 5. Darney PD, Klaisle CM Tanner S, et al: Curr Probl Obstet Gynecol Fertil 1990;13:89-125.

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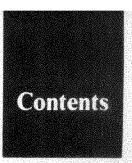
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December Part 1

CONSULTANTS

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1579

PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION

Elevated fetal plasma lactate produces polyhydramnios in the sheep

1595

Theresa L. Powell, PhD, and Robert A. Brace, PhD

San Diego, California

Intravenous infusion of sodium lactate into near-term fetal sheep for 3 days resulted in polyhydramnios but not hydrops fetalis.

Left thoracic duct lymph flow responses to angiotensin II or atrial natriuretic factor infusion in the ovine fetus

1607

Robert A. Brace, PhD, and Robert L. Andres, MD

San Diego, California

The current study suggests that angiotensin II may stimulate and atrial natriuretic factor may suppress lymphatic function in the ovine fetus.

Oral-nasal membranes are not the major route for fetal absorption of amniotic fluid arginine vasopressin

1614

William M. Gilbert, MD, Cecilia Y. Cheung, PhD, and Robert A. Brace, PhD San Diego, California

The ovine fetal oral-nasal membranes do not appear to be a route of significant absorption of intraamniotically injected arginine vasopressin relative to absorption by the intramembranous pathway.

Amniotic fluid volume response to esophageal occlusion in fetal sheep

1620

Yuji Fujino, MD, Connie L. Agnew, MD, Peter Schreyer, MD, M. Gore Ervin, PhD, Dan J. Sherman, MD, and Michael G. Ross, MD

Torrance, California

Esophageal occlusion of fetal sheep resulted in a threefold increase in amniotic fluid volume.

(Contents continued on page 6A)

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Atrial natriuretic factor responses to volume expansion in pregnant and nonpregnant sheep

1627

Timothy L. Bennett, MD, and James C. Rose, PhD Kansas City, Kansas, and Winston-Salem, North Carolina

Atrial natriuretic factor responses to isotonic volume expansion are similar in pregnant and nonpregnant sheep. Atrial pressure and urine flow changes are unaltered by pregnancy.

Fetal and maternal plasma atrial natriuretic factor responses to angiotensin II infusion

1635

Michael G. Ross, MD, John P. Cardin, MD, Lony Castro, MD, M. Gore Ervin, PhD, and Rosemary D. Leake, MD

Torrance and Los Angeles, California

In spite of similar absolute increases in systemic blood pressure, angiotensin II infusion stimulated increased fetal, although not maternal, plasma atrial natriuretic factor levels.

Angiotensin II vascular smooth-muscle receptors are not down-regulated in near-term pregnant sheep

1641

Hasu R. Mackanjee, MB, ChB, Philip W. Shaul, MD, Ronald R. Magness, PhD, and Charles R. Rosenfeld, MD Dallas, Texas

Although plasma angiotensin II increases threefold to fourfold during ovine pregnancy, receptor density and affinity in vascular smooth muscle from aorta or uterine and mesenteric arteries are unaltered.

(Contents continued on page 9A)

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Cefotan arithmetic.



more cost-effective than cefoxitin^{1,2}



less frequent dosing than cefoxitin^{3,4}



several times the half-life of cefoxitin^{5,6}

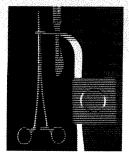


single-dose prophylaxis/ twice-daily treatment



References: 1. Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. Am J Surg. 1988; 155(5A):101-102. 2. Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cerombination antibiotic regimens. Am J Surg. 1988;155(5A):96-101. 3. CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. 4. Physicians' Desk Reference. ed 43. Oradell, NJ: Medical Economics Co.; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. 5. Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. Infect Surg. April 1986 (suppl), pp 11-14. 6. Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. Am J Surg. 1988;155(5A):67-70. 7. Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. MMVM. 1989;38(suppl 5-8):1-43.

Please see adjacent page for brief summary of prescribing information.



Count on FOTA

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use (FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.)

INDICATIONS AND USAGE

INDICATIONS AND USAGE
Treatment: CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary fract Infections caused by E coli, Klebsiella species (including K pneumoniae), Proteus mirabilis, and Proteus so (which may include the organisms now called Proteus vuigaris, Providencia rettgeri, and Morganella morganii).
Lower Respiratory Tract Infections caused by Streptococcus pneumoniae (formerly D pneumoniae), Staphylococcus aureus (penicillinase- and nonpenicillinase- producing strains), Haemophilus influenzae (including ampicillin-resistant strains), Klebsiella species (including K pneumoniae), E coli, Proteus mirabilis and Serratia marrescens:

Lower Respiratory Tract Infections caused by Streptococcus pneumoniae (formerly D pneumoniae), Staphylococcus aureus (penicillinase-and nonpenicillinase-producing strains), Haemophilus influenzae (including ampicillin-resistant strains), Klebsiella species (including K pneumoniae), E coli, Proteus mirabilis, and Serratia marcescens:

Skin and Skin Structure Infections caused by Staphylococcus aureus (penicillinase-and nonpenicillinase-producing strains), Staphylococcus epidermidis, Streptococcus species (excluding enterococci), E coli, Klebsiella pneumoniae, and Peptococcus: Species (excluding enterococci), E coli, Klebsiella pneumoniae, and Peptococcus species (excluding enterococci), group is streptococcus, Staphylococcus aureus (including penicillinase-and nonpenicillinase-producing strains), Staphylococcus apidermidis, Streptococcus species (excluding enterococci), group is streptococci, E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides species (excluding distasonis, B ovatus, and B thetaiotaomicron), Fusobacterium species, and gram-positive anaerobic cocci (including Peptococcus and Peptosteptococcus species).

Intra-abdominal Infections caused by E coli, Klebsiella species (including K pneumoniae), Streptococcus species (excluding metrococci), Bacteroides species (excluding B distasonis, B ovatus, and B thetaiotaomicron), and Clostridium species.

Bone and Joint Infections caused by Staphylococcus aureus.*

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known, however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has n

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known aliergy to the cephalosporin group of antibiotics.

WARNINGS

Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures. Pseudomembranous colitics has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may after normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by Clostridium difficile is one primary cause of antibiotic-associated colitis.

Mild cassociated to say respond to drug discontinuance alone. Moderate to severe cases should be

antibiotic-associated colitis.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by Calificile. Other causes should also bensidered. In common with many other broad-spectrum antibiotics, CEFUTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

PRECAUTIONS
General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachyoardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution. As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of

Seroin and unite creaming seven by safe reaction and produce raise increases in the local of creatining reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of

500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought t developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicul weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatog and spermatocytes; Sertoli and Leydig cells were unaffected, incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 tre animals was affected, and the degree of degeneration was mild. Similar lesions have been observed in experiments of comparable design with other methylthiotetra: containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks iniffant dops (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertilit harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

ADVENSE REALFIUNS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80):

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) : nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 2 positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGO (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injectio (1 in 200), and discomment (1 in SGO).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injectio (1 in 300), and discomfort (1 in 500).

During postmarketing experience with CEFOTAN, agranulocytosis, anaphylactic reactions, fever, hemolytic anemia, leukopenia, prolonged prothrombin time with or without bleeding, pseudomembrar colitis, and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

Treatment: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intra-muscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be de mined by the condition of the patient, severity of the infection, and susceptibility of the causative organ

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN				
Type of infection	Dally Dose	Frequency and Route		
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM		
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM		
Severe	4 grams	2 g every 12 hours IV		
Life-Threatening	6* grams	3 g every 12 hours IV		

*Maximum daily dosage should not exceed 6 grams.

Prophylaxis: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenousl 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be admistered as soon as the umbilical cord is clamped.

Impaired Renal Function: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION Creatinine Clearance Dose mL/mln Frequen >30 Usual Recommended Dosage Every 12 h 10-30 Usual Recommended Dosage Every 24 h Usual Recommended Dosage Every 48 h <10

*Dose determined by the type and severity of infection, and susceptibility of the causative organism Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/l When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

Males:

Weight (kg) × (140 - age) 72 × serum creatinine (mg/100 mL)

Females:

0.9 x value for males

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

Manufactured for



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The peak rectal temperature attained during running and aerobic dance falls progressively

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The maternal concentration ratios of vitamin E to lipid peroxides and prostacyclin to thromboxane progressively favor the antioxidant activity of vitamin E and the vasodilating actions of prostacyclin with advancing gestation in normally pregnant women.

The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood

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Yuping Wang, MD, Scott W. Walsh, PhD, Jingde Guo, MD, and Junyan Zhang, MD Richmond, Virginia, and Harbin, People's Republic of China

There is an imbalance of increased lipid peroxides and decreased vitamin E in preeclampsia that correlates with the imbalance of increased thromboxane and decreased prostacyclin.

Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension

1701

Stephen J. Wisdom, MB, ChB, Rhoda Wilson, PhD, James H. McKillop, PhD, and James J. Walker, MB, ChB Glasgow, Scotland

Antioxidant systems in nonpregnant women, pregnant women with normal blood pressure, and women with pregnancy-induced hypertension were compared, with implications for the role of oxidative stress in these conditions.

Selective effects of preeclamptic sera on human endothelial cell procoagulant protein expression

1705

Robert N. Taylor, MD, PhD, David C. Casal, PhD, Lynn A. Jones, MA, Madhu Varma, PhD, James N. Martin, Jr., MD, and James M. Roberts, MD

San Francisco and Sunnyvale, California, and Jackson, Mississippi

Monolayer cultures of human endothelial cells exposed to sera from patients with preeclampsia released more cellular fibronectin than cells treated with sera from matched pregnant controls, but other procoagulant activities were not differentially affected.

Potential role of endothelin-1 in normal and hypertensive pregnancies

1711

Dimitrios S. Mastrogiannis, MD, PhD, William F. O'Brien, MD, Judith Krammer, MD, and Ray Benoit, BS Tampa, Florida

Endothelin-1 concentration is elevated in preeclampsia, laboring patients, and umbilical venous blood.

Endothelin-1-induced vasoconstriction is not mediated by thromboxane release and action in the human fetal-placental circulation

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Leslie Myatt, PhD, Gretchen Langdon, BA, Anthony S. Brewer, BS, and Diane E. Brockman, MS Cincinnati, Ohio

Endothelin-1 is a potent vasoconstrictor of the human fetal-placental circulation, but its action is not mediated by thromboxane release or action.

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An endothelial cell model for the investigation of the molecular regulation of fetal vascular tone

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Donald J. Dudley, MD, Sharie LaMarche, BS, and Murray D. Mitchell, DPhil Salt Lake City, Utah

Endothelial cell prostacyclin production is regulated by inflammatory cytokines, protein kinase C agonists, and calcium ionophores in a concentration-related fashion.

In utero diagnosis of congenital varicella zoster virus infection by chorionic villus sampling and polymerase chain reaction

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Nelson B. Isada, MD, David P. Paar, MD, Mark P. Johnson, MD, Mark I. Evans, MD, Wolfgang Holzgreve, MD, Faisal Qureshi, MD, and Stephen E. Straus, MD

Detroit, Michigan, Münster, Germany, and Bethesda, Maryland

Polymerase chain reaction identified varicella zoster virus-specific genome material in placental tissue by chorionic villus sampling in two patients with chickenpox.

Prenatal diagnosis with fetal cells isolated from maternal blood by multiparameter flow cytometry

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James O. Price, PhD, Sherman Elias, MD, Stephen S. Wachtel, PhD, Katherine Klinger, PhD, Michael Dockter, PhD, Avirachan Tharapel, PhD, Lee P. Shulman, MD, Owen P. Phillips, MD, Carole M. Meyers, MD, Donna Shook, MS, and Joe Leigh Simpson, MD

Memphis, Tennessee, and Framingham, Massachusetts

Fetal cells were sorted from maternal blood for prenatal diagnosis of fetal sex, trisomy 21, and trisomy 18.

Absence of hyperinsulinemia in isoimmunized fetuses treated with intravascular transfusion

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Michael L. Socol, MD, Sharon L. Dooley, MD, Judith A. Ney, MD, John P. Minogue, DMin, Dietra D. Millard, MD, and Edward S. Ogata, MD

Chicago, Illinois

We were unable to demonstrate hyperinsulinemia in serial blood samples from isoimmunized fetuses treated with intravascular intrauterine transfusion.

Does the brachial artery Doppler flow velocity waveform reflect changes in downstream impedance?

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Fiona M. Fairlie, MRCOG, and James J. Walker, MD Glasgow, Scotland

Maternal brachial artery pulsatility index and peak systolic and end-diastolic frequencies were related to changes in downstream impedance, cardiac output, and heart rate in uncomplicated pregnancies.

Effect of pregnancy on the accuracy of light-reflection rheography

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John R. Allbert, MD, William E. Roberts, MD, L. Wayne Hess, MD, and John C. Morrison, MD Jackson, Mississippi

The performance of light-reflective rheography used to detect venous thrombosis does not seem to be affected by pregnancy.

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Anticardiolipin antibody-positive serum enhances endothelial cell platelet-activating factor production

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Richard K. Silver, MD, Luba Adler, MS, Andrew R. Hickman, and Joseph R. Hageman, MD Evanston, Illinois

Anticardiolipin antibodies may promote thrombosis by enhancing the synthesis of platelet-activating factor within vascular endothelial cells.

Combination antibiotics and indomethacin in idiopathic preterm labor: A randomized double-blind clinical trial

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Edward R. Newton, MD, Laurence Shields, MD, Louis E. Ridgway III, MD, Michael D. Berkus, MD, and Byron D. Elliott, MD

San Antonio, Texas

The use of ampicillin-sulbactam plus indomethacin did not prevent magnesium sulfate tocolysis failure and subsequent preterm birth.

The progesterone antagonist onapristone increases the effectiveness of oxytocin to produce delivery without changing the myometrial oxytocin receptor concentrations

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Krzysztof Chwalisz, MD, PhD, Falk Fahrenholz, PhD, Mario Hackenberg, Robert Garfield, PhD, and Walter Elger, MD, PhD

Berlin and Frankfurt, Germany, and Hamilton, Ontario, Canada

The uterine reactivity to oxytocin was enhanced by onapristone; there was no increase in oxytocin receptors, but a substantial elevation in gap junctions in the myometrium was noted.

Dose-related action of gonadotropin-releasing hormone on basal prostanoid production from the human term placenta

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Inn Soo Kang, MD, Mi Kyoung Koong, MD, Julie Forman, BS, and Theresa Marie Siler-Khodr, PhD San Antonio, Texas

Gonadotropin-releasing hormone may have a physiologic role in regulating placental prostanoids by inhibiting their production in a dose-dependent fashion.

Circadian myometrial and endocrine rhythms in the pregnant rhesus macaque: Effects of constant light and timed melatonin infusion

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Toshihiko Matsumoto, MD, David L. Hess, PhD, Kanchan M. Kaushal, MS, Guillermo J. Valenzuela, MD, Steven M. Yellon, PhD, and Charles A. Ducsay, PhD Loma Linda, California, and Beaverton, Oregon

Twenty-four-hour patterns in uterine activity and steroid secretion in pregnant rhesus monkeys were maintained in constant light, indicating endogenous circadian rhythms.

Effects of intravenous cocaine on reproductive function in the mated rabbit

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Susan J. Atlas, PhD, and Edward E. Wallach, MD Baltimore, Maryland

A single daily intravenous cocaine injection increased ovulation in mated rabbits, although six doses administered immediately after mating delayed preimplantation development but not implantation.

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Paul Bischof, PhD, Evelyne Friedli, MSc, Marzia Martelli, MSc, and Aldo Campana, MD Geneva, Switzerland

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Improvement of in vitro fertilization and early embryo development in mice by coculture with human fallopian tube epithelium

1802

Jeffrey M. Goldberg, MD, Essam Al-Dein M. Khalifa, MD, Chad I. Friedman, MD, and Moon H. Kim, MD Columbus, Ohio

Human fallopian tube epithelium explants cocultured with a murine in vitro fertilization model increased fertilization and blastocyst transformation rates over those of conditioned and unconditioned media.

Comparison of intermittent and continuous use of a gonadotropinreleasing hormone antagonist (Nal-Glu) in in vitro fertilization cycles: A preliminary report

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Denise L. Cassidenti, MD, Mark V. Sauer, MD, Richard J. Paulson, MD, Edward C. Ditkoff, MD, Jean Rivier, PhD, Samuel S.C. Yen, MD, DSc, and Rogerio A. Lobo, MD Los Angeles, California

The intermittent use of the gonadotropin-releasing hormone antagonist Nal-Glu for in vitro fertilization significantly decreases cycle length and blocks spontaneous luteinizing hormone surges without compromising oocyte and embryo yield when compared with the gonadotropin-releasing hormone agonist.

The gonadotropin-releasing hormone antagonist (Nal-Glu) acutely blocks the luteinizing hormone surge but allows for resumption of folliculogenesis in normal women

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Edward C. Ditkoff, MD, Denise L. Cassidenti, MD, Richard J. Paulson, MD, Mark V. Sauer, MD, Wellington L. Paul, PhD, Jean Rivier, PhD, Samuel S.C. Yen, MD, DSc, and Rogerio A. Lobo, MD Los Angeles and Van Nuys, California

Nal-Glu acutely inhibits the luteinizing hormone surge and arrests ovulation but allows follicular rescue with subsequent ovulation and a normal luteal phase.

Reduction of primary posttraumatic adhesion formation with the prostacyclin analog iloprost in a rodent model

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Alex Steinleitner, MD, Hovey Lambert, PhD, Mariluz Suarez, BS, Nurys Serpa, BS, and Beverly Robin, BS Miami Beach and Miami, Florida

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Sanford M. Markham, MD, Norman H. Dubin, PhD, and John A. Rock, MD Baltimore, Maryland

Platelets from women are more sensitive than platelets from men to arachidonic acid aggregation; this difference is not affected by decompression stress.

Tryptophan and neutral amino acids in premenstrual syndrome

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Andrea J. Rapkin, MD, Anthony E. Reading, PhD, Stephanie Woo, BA, and Linda M. Goldman, RNP, MN Los Angeles, California

The ratio of tryptophan to amino acids that compete for transport across the blood-brain barrier was similar in subjects with premenstrual syndrome and controls.

The role of prostaglandins in detrusor instability

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Arieh Bergman, MD, Frank Z. Stanczyk, PhD, and Rogerio A. Lobo, MD Los Angeles, California

Women with detrusor instability had significant reductions in the production of 6-keto prostaglandin $F_{1\alpha}$ and no difference in the production of prostaglandin $F_{2\alpha}$ or thromboxane B_2 when compared with women who did not have detrusor instability.

Is 11β -hydroxyandrostenedione a better marker of adrenal androgen excess than dehydroepiandrosterone sulfate?

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Frank Z. Stanczyk, PhD, Lilly Chang, MD, Enrico Carmina, MD, Zdnek Putz, CSc, and Rogerio A. Lobo, MD Los Angeles, California, Palermo, Italy, and Lubochna, Czechoslovakia

 11β -hydroxyandrostenedione is a useful marker of adrenal androgen secretion; the greater sensitivity of 11β -hydroxyandrostenedione over dehydroepiandrosterone sulfate to adrenal stimulation and suppression suggests its unique diagnostic use.

Effects of peritoneal macrophages from patients with endometriosis on the proliferation of endometrial carcinoma cell line ECC-1

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Ren-jie Zhang, MD, Robert A. Wild, MD, Diane Medders, BS, and Subba Rao Gunupudi, PhD Oklahoma City, Oklahoma, and Sunnyvale, California

Cytokines from peritoneal macrophages in patients with endometriosis may play an important role in the progression of the disease, and such an effect may be mediated by epidermal growth factor or a related growth factor.

Prolonged clearance of intraperitoneal $16\alpha[^{125}I]iodo-17\beta$ -estradiol in presence of ascites

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Anton Scharl, MD, Stig Kullander, MD, Matthias W. Beckmann, MD, Jay A. Spicer, MS, Richard J. Baranczuk, PhD, and John A. Holt, PhD

Chicago, Illinois, Malmø, Sweden, and Kansas City and Overland Park, Kansas

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Lauren J. Dungy, MD, Tariq A. Siddiqi, MD, and Sohaib Khan, PhD Cincinnati, Ohio

The temporal expression of c-jun and jun-B in human placentas may be related to the proliferation and differentiation of trophoblastic cells.

New monoclonal antibodies identify the glycoprotein carrying the CA 125 epitope

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Timothy J. O'Brien, PhD, Lawrence M. Raymond, PhD, Gary A. Bannon, PhD, Danny H. Ford, Hildur Hardardottir, MD, Frank C. Miller, MD, and J. Gerald Quirk, Jr., MD, PhD Little Rock and Fayetteville, Arkansas

New antibodies identify epitopes on the CA 125 glycoprotein similar to and distinct from OC 125.

Specific binding sites for insulin and insulin-like growth factor I in human endometrial cancer

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Manubai Nagamani, MD, Charles A. Stuart, MD, Patricia A. Dunhardt, BA, and Mark G. Doherty, MD Galveston, Texas

Specific binding sites for insulin and insulin-like growth factor I are present in human endometrial cancer, and insulin and insulin-like growth factor I may play a role in the growth and development of endometrial cancer.

Increased progesterone concentrations are necessary to suppress interleukin-2-activated human mononuclear cell cytotoxicity

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Bruce B. Feinberg, MD, Nenita S. Tan, MD, Bernard Gonik, MD, Peter C. Brath, BS, and Scott W. Walsh, PhD Houston. Texas

The higher placental production of progesterone seen in preeclampsia may be a trophoblast compensatory response to immunoactivated maternal effector cells.

Sister chromatid exchange frequency in directly prepared cytotrophoblasts: Demonstration of in vivo deoxyribonucleic acid damage in pregnant women who smoke cigarettes

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Lee P. Shulman, MD, Sherman Elias, MD, Avirachan T. Tharapel, PhD, Lirong Li, PhD, Owen P. Phillips, MD, and Joe Leigh Simpson, MD

Memphis, Tennessee

Cytotrophoblasts from women who smoke cigarettes during pregnancy exhibit increased frequency of sister chromatid exchange, indicating direct placental deoxyribonucleic acid damage.

Cellular localization of müllerian inhibiting substance messenger ribonucleic acid during human ovarian follicular development

1881

Gail F. Whitman, MD, and Cooley G. Pantazis, MD Augusta, Georgia

Localization of reaction product within the cytoplasm demonstrates that active transcription of müllerian inhibiting substance messenger ribonucleic acid occurs within both the fetal and the postnatal human ovary.

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The presence of the testicular determining sequence, SRY, in 46,XY females with gonadal dysgenesis (Swyer syndrome)

1887

M. Ali Behzadian, PhD, Sandra P.T. Tho, MD, and Paul G. McDonough, MD Augusta, Georgia

The deoxyribonucleic acid sequence coding for the functional domain of the candidate gene for testicular determination factor, SRY, is intact in 46,XY females with pure gonadal dysgenesis (Swyer syndrome).

Evidence for a partial deletion in the androgen receptor gene in a phenotypic male with azoospermia

1891

James W. Akin, MD, Ali Behzadian, PhD, Sandra P.T. Tho, MD, and Paul G. McDonough, MD Augusta, Georgia

Molecular analysis of the androgen receptor gene revealed a partial deletion in the deoxyribonucleic acid of one of seven azoospermic patients studied.

LETTERS TO THE EDITORS

Giacomello's observation and nuchal cords Jason Collins, MD Slidell, Louisiana 1895

Reply Francesco Giacomello, MD Rome, Italy 1895

Ethics in medical studies with human subjects David M. Sherer, MD, and Jacques S. Abramowicz, MD Rochester, New York 1895

Reply Israel Goldstein, MD, and Etan Zimmer, MD Haifa, Israel 1896

Factors affecting embryo implantation after human in vitro fertilization David R. Meldrum, MD Redondo Beach, California 1896

Reply Richard J. Paulson, MD, and Mark V. Sauer, MD Los Angeles, California 1896

The origin of brain lesions in survivors of twin gestations complicated by fetal death Ron Gonen, MD Haifa, Israel 1897

First report: Prenatal diagnosis of a true knot Jason H. Collins, MD Slidell, Louisiana 1898

The effect of amnioinfusion on uterine pressure and activity Fred S. Miyazaki, MD Los Angeles, California 1898

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Reply Michael A. Finan, MD Tampa, Florida 1899

Alternative estimates of ectopic pregnancy risks during contraception Irving Sivin, MA New York, New York 1899

Reply Adele L. Franks, MD, Valerie Beral, MB, BS, Willard Cates, Jr., MD, MPH, and Carol J.R. Hogue, PhD Atlanta, Georgia 1900

First report: Prenatal diagnosis of long cord Jason Collins, MD Slidell, Louisiana 1900

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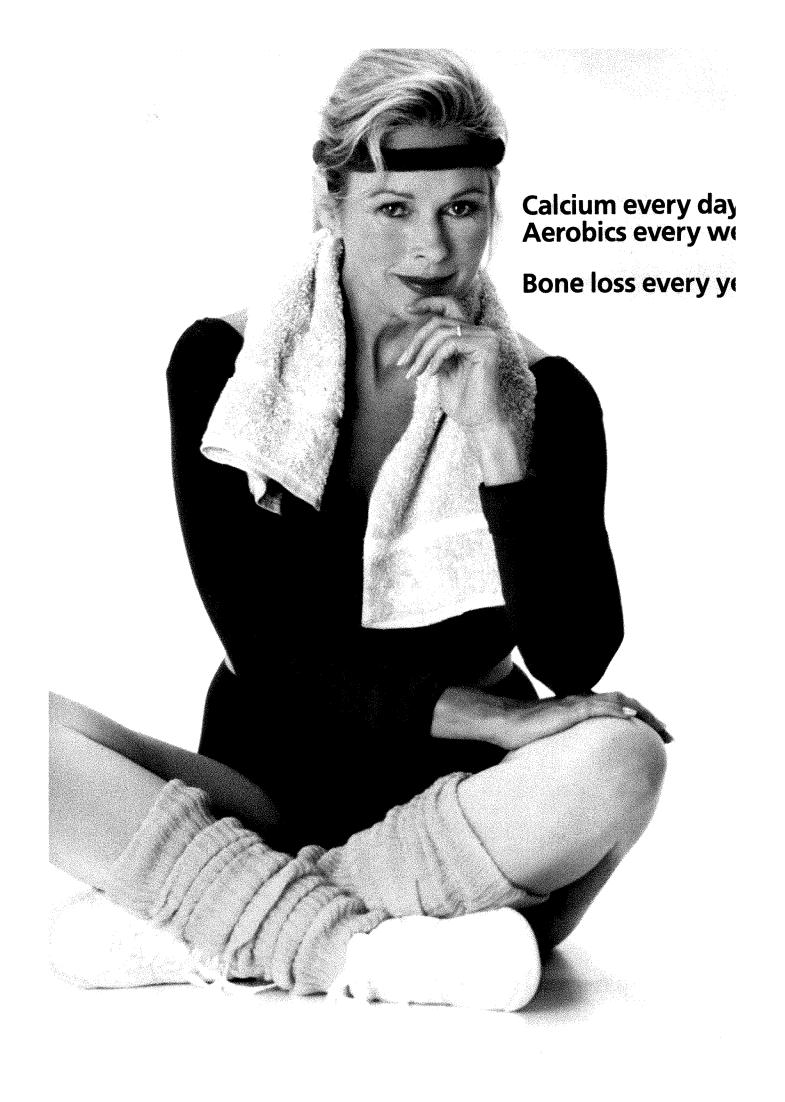
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Editors' note

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

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Calcium¹ and exercise are not enough to prevent postmenopausal osteoporosis, since estrogen deficiency is the primary cause.² PREMARIN is the only brand of estrogen indicated to prevent further bone loss and in a recently published study has been shown to reduce the risk of hip fractures by as much as 66%.³

Early PREMARIN therapy offers the best protection

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Protection continues as long as estrogen therapy continues

Clinical studies show that within three years of menopause when therapy is withdrawn, bone loss begins again.4

PREMARIN® (conjugated actrogens tablets)

(conjugated estrogens tablets)

Proven benefits for menopause and beyond

Estrogens have been reported to increase the risk of endometrial carcinoma in postmenopausal women. Estrogens should not be used in women (or men) with any of the following conditions: known or suspected 1) pregnancy, 2) breast cancer, 3) estrogendependent neoplasia, 4) undiagnosed abnormal genital bleeding, 5) active thrombophlebitis or thromboembolic disorders.

For moderate-to-severe vasomotor symptoms and for osteoporosis

PREMARIN

(conjugated estrogens tablets)











0.3 mg 0.625 mg 0.9 mg 1.25 mg

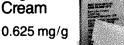
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For atrophic vaginitis

PREMARIN'

(conjugated estrogens)

Vaginal Cream





BRIEF SUMMARY (FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE

PREMARIN® Brand of conjugated estrogens tablets, USP PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquelying base

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal biteding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

2 ESTROGENS SHOULD NOT BE USED DURING PREGNANCY. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and temale fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.

There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares urine. It contains estrone, equilin, and 17 α -dihydroequilin, together with smaller amounts of 17 α -estradiol, equitenin, and 17 α -dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens.

estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and frautresis within

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and karurosis vulvae.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and karurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women for men) with any of the following conditions:

1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients. WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen epiacement doses. Endomentical cancer risk some estrogen dose. In patients on combined estrogen-progestin therapy this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of telal congenital reproductive tract disorders.

Estogen thetapy utiling preglaricy is associated with an incleased risk of rear congeniar reproductive had disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose. Blood pressure should be monitored with estrogen use, especially if high doses are used. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECALTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial mularation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should

include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin

The following drug/laboratory test interactions have occurred to the combinations (oral contraceptives):

1. increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

2. increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T4, levels determined by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaftered.

reflecting the elevated 18G; free 1₂ concentration is unaftered.

3. Impaired glucose tolerance.

4. Reduced response to metyrapone test.

5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See CONTRAINDICATIONS and Royed Warning.

PREGNARCY CATEGORY X: Estrogens should not be used during pregnancy. See CONTRAINDICATIONS and Boxed Warning.

MURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine libromyomata, vaginal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scatp hair, insultism; steepening of corneal curvature, intolerance to contact lenses, headache, migraine, dizziness, mental depression, chorea: increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated strogens tablets, USP

1. Given cyclically for short-term use only. For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic vertificts associated with the menogause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be expelic (eg. three weeks on and one week off). Attempts to discontinue or taper medication should be made at three-to is isk-month intervals.

2. Given cyclically: Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. It bleeding does not occur by the end of this period, the same dosage schedule according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg. th

CONITOL.

Osteoporosis—0 625 mg daily. Administration should be cyclic (eg. three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Altempts to discontinue or taper medication should be made at three- to six-month intervals. Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition. Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989.

Revised August 21, 1989

Heterences:1. Nilas L, Christiansen C, Røddro P, Calcium supplementation and postmenopausal bone loss. *Br Med J* 1984. 289:1103-1106. 2. Riggs BL. Research Directions in Osteoporosis. Scientific Workshop, National Institutes of Health, February 9-11, 1987. 3. Kiel DP, Felson DT. Anderson JJ, et al. Hip fracture and the use of estrogens in postmenopausal women. *N Engl. J. Med.* 1987; 317:1169-1174. 4. Christiansen C. Christensen MS. Transbel I Bone mass in postmenopausal women after withdrawal of cestrogen/gestagen replacement therapy. *Lancet* 1981; 1459-461.



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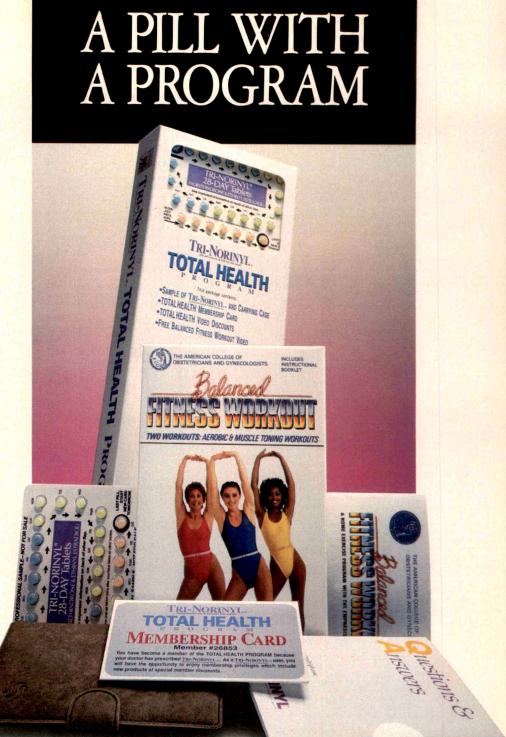
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The TRI-NORINYL Total Health Program sample package contains one cycle of TRI-NORINYL; the attractive Wallette® Pill Dispenser; and a patient Q&A booklet—plus the video and instruction guide, The ACOG Balanced Fitness Workout; and program membership.

The TRI-NORINYL Total Health Program provides a variety of fitness and health information to help women achieve healthier lifestyles.

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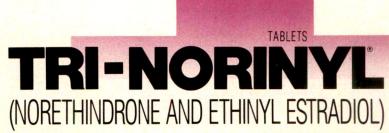
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Serious as well as minor side effects have been reported following the use of oral contraceptives. These include thromboembolic disease.

Please see adjacent page for brief summary of prescribing information.

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ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethnyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

REMONEY 28-DAY Sablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet con-ratins norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Throm bophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders . Cerebral vascular or coronary artery disease . Known or suspected accinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of preg-nancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contra ceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, benatic peoplasia and gallbladder including impoculation and uncontrolled in the processing storage and particularly discussed and particularly discussed in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholes terulemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estro-gen. The effects of long-term use with lower dose formulations of both estrogens and progesto-gens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective of integrated this tables general general magnets account separate are in two types: reconspective or case control studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the ratio of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control. studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not prosatures, but the bods and obtained a descendent relative that are a feet leaf to the descending the individual of the feet leaf to the feet le

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

 Myocardial infarction
 An increased risk of myocardial infarction has been attributed to oral contraceptive use. This An increased risk of importantal infarction has been actituded to did contraceptive base. This first is primarily in smokers or women with other underlying risk factors for coronary artery dis-ease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use ora contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obe-sity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Smil ar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis. 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of throm boembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2 to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discon-tinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke, thypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

in a large study, the relative risk of thombotic strokes has been shown to range from 3 for nor-motensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use raci contraceptives, 7.6 for smokers who used oral contraceptives, 1.6 nor-motensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density [popor etnis (HDL) has been reported with some progestational agents. A decline in serum high den-sity [poporteins has been associated with an increased incidence of ischemic heart disease Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of

therapeutics. For any particular estrogen/progestogen combination, the dosage regimen pre scribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e Persistence of risk of vascular disease

e. Persistence of risk of vascular disease There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40–49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of roal contracep-tives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives, However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

2. ESTIMATES OF MORTHLITT FROM CONTINUED IN TRACE TIVE 20 One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific bene fits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970s—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lover estrogen dose formulations combined with careful restriction of oral contra-ceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which sug-gest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Ferthifty and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular dis asked or televine to plan in 150. The commerce common that shoops of the 150 the commerce cases are asked to the common that the common that the common that the common that the common that the common that the common that the alternative surgical and medical risks associated with pregnancy in older women and with the alternative surgical and medical risks during the common that the alternative surgical and medical risks associated with pregnancy in older women of the alternative surgical and medical risks associated with pregnancy in older that the alternative surgical and medical risks associated with pregnancy in the common that the alternative surgical and medical risks associated with pregnancy in the common that the comm means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE URBANS.

Numerous epidemiological studies have been performed on the incidence of breast, endome trial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the reketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect. neered oranius and uoses. The Cancer and Steriou formome study also Snowed in Diatent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in examination of users and non-users and difference in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there con-tinues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established

4. HEPATIC NEOPLASIA

Renign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 33 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing benatocel Suddies in the United States and Britain have shown an increased risk of developing regulations fulfar carcinoma in long-term (> 8 years) oral confraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver can cers in oral confraceptive users is less than 1 per 1,000,000 users.

5 OCHLAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral con traceptives. Oral contraceptives should be discontinued if there is unexplained partial or com plete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratagenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contracentives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threat ened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnance should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Transcription of the description recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

a. Carbon Turkal Evaluation Train and Evaluation effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and dia betic women should be carefully observed while taking oral contraceptives

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed ear lier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The inci-All indicates in about personner has been reported in mornier manifestor among other women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens. Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contracept tives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure with return to normal after stopping gral contraceptives and there is no difference in the occurrence nsion among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause

11 RIFFRING IRREGILLARITIES

II. DECEVING IRREBULARITIES
Breakthrough bleeding and spotting are sometimes encountered in patients on oral contracep-tives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amen-orrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdo men and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic meas-ures should be conducted for ule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2 LIPIN DISORDERS

2. LITHO DISORDERS Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral confraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregular-ties have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenyloin sodium, and possi-bly with grisedfulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased

norepinephrine-induced platelet aggregability.

b. Increased thryoid binding globulin (186) leading to increased circulating total thryoid hormone, as measured by protein-bound oidine (18b), 1/4 by column or by radioimmunoassay. Free 13 resin uptake is decreased, reflecting the elevated 18G. Free 14 concentration is unaltered.

c. Other binding proteins may be elevated in serum.
d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids, however, free or biologically active levels remain unchanged.
e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum foldte levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

10. PREGNANCY Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections.

Pregnancy Lategory 3. See CUNINAINDICATIONS and WARNINGS Sections.

TI. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weared her child.

INFORMATION FOR THE PATIENT

See PATIENT LABELING. ADVERSE REACTIONS

An increased risk of the following senious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section). Thrombophiebitis - Arterial thromboembolism - Pulmonary embolism - Myocardial infarction - Cerebral hemorrhage - Cerebral thrombosis - Hypertension - Gallibladder disease - Hepatic adenomas, carcinomas or benign liver

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis . Reti nal thromhosis

nal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal carmps and bloading) - Breakthrough bleeding • Sopting • Change in menstrual flow - Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminipton in lection when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Menal Indigensions • Refund for Jerosch or Largeburkrafes • Alexange and Griess • Change in covered to describe the second of the contraction of the second of the contraction of t tal depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in cornea

tal depression • Neduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal
curvature (Stepening) • Intolerance to contact lenses.
The following adverse reactions have been reported in users of oral contraceptives and the
association has been entheir confirmed nor refuted: Pre-menstrual syndrome • Cataracts •
Changes in appetite • Cystitis-like syndrome • Nedadoche • Nervousness • Dizziness • Hirsutsim • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Nemorrhagic eruption •
Vaginits • Porphyra: • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colifis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral con-traceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol. Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the treast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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The original and two good-quality photocopies of the manuscript and three sets of black-and-white glossy prints of illustrations are required. See "Color figures" and "Computer-generated figures" for special requirements. Manuscripts must be submitted in English.

Manuscripts must be typed double-spaced on one side only of 22×28 cm ($8\frac{1}{2} \times 11$ inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, figure legends, and tables.

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Text. Do not hesitate to write your manuscript in the first person and active voice if they are more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the Journal. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a Journal page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

Council of Biology Editors Style Manual or the AMA's Manual of Style. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify *Material and Methods*, *Results*, and *Comment*. Authors may wish to summarize their findings in a short paragraph at the end of the *Comment* section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to repeat the study) the plan, the patients, experimental animals or other species, material, and controls, the methods and procedures utilized, and the statistical method(s) employed.

In the Results section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the *Results* section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

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For black-and-white figures, submit three sets of 3×4 inch (minimum) to 5×7 inch (maximum) unmounted, glossy prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy prints with good black-and-white contrast.

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Triphasil

Levonorgestrel and ethinyl estradiol tablets— Triphasic regimen 21- and 28-day regimens



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Simple, easy-to-use Day 1 Start

Patient acceptance proven over time

 Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.
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THE OC TO START WITH BECAUSE SHE'LL STAY WITH IT

IN BRIEF: TRIPHASIL IN BRIEF:

TRIPHASILE* — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol; 17 light-green tablets containing inert ingredients are included in the 28-day regimen) — Triphasic regimen.

Indications and Usage — TRIPHASILE* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Oral contained captives (COS) as a measure or Contrained property of the following: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Endometrial carcinoma or other known or suspected storpen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding, 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected description. Known or suspected pregnancy.

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contra-ceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. Use of OCs is associated with increased risks of serious conditions including myocardial infarction, thrombo-

Use of OCs is associated with increased risks of serious conditions including myocardial infarction, thrombomboiling, stroke, hepatic neoplasia, galibladder disease, and hypertension, although risk of serious morbiolity/mortality is very small in healthy women without underlying risk factors. Morbidity/mortality risk increases significantly in other risk factors preservising OCs should be familiar with the following information relating to these risks. (This information is based principally on data involving OCs with higher doses of estrogen and progestogen than those commonly used today. Effect of long-term use of lower estrogen and progestogen formulations is yet to be determined.)

1. Thromboemboilic Disorders and Other Vascular Problems — MYOCARDIAL, INFARCTION (MI). An increased risk of MI has been attributed to OC use. Risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease (i.e. hypertension, hypercholesterolemia, morbid obesity, diabetes). Relative risk of heart attack for current OC users is estimated to be two to six risk is very low under the age of Users. Smoking combined with OC uses on the understance substantially is incidence of Mis in women in their mid-thirties or older with smoking accounting for majority of excess cases. Mortality rates associated with circulatory disease increase substantially in smokers over the age of 40 among OC users. OCs may compound effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. OCs have been shown to increase blood pressure among users (see Warnings). Similar effects or wisk factors are associated with increased risk of heart disease. Use OCs with caution in women with cardiovascular disease risk factors.

THROMBOEMBOLISM. Increased risk of thromboembolic and thrombotic disease associated with OC use is well established. In cas

due to OCs is not related to length of use and disappears after pill use is stopped.

A 2- to 4-fold increase in relative risk of postoperative thromboembolic complications has been reported with OCs. Relative risk of venous thrombosis in women with predisposing conditions is twice that of women without such conditions. If feasible, discontinue OCs at least 4 weeks prior to and for 2 weeks after elective surgery of a type associated with increased risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is associated with an increased thromboembolic risk, start OCs no earlier than 4 to 6 weeks after delivery in women not breast-feeding, or a mid-trimester pregnancy termination. CEREBROVASCULAR DISEASES. OCs increase relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes); in general, risk is greatest among older (> 35 years), hypertensive women who smoke. Hypertension is a risk factor for users and nonusers, for both types of strokes, while smoking interests in circases hemorrhagic stroke is.

who smoke. Hyperfension is a risk factor for users and nonusers, for both types of strokes, while smoking interacts to increase hemorrhagic stroke risks. ROSE-RELATED RISK OF VASCILLAR DISEASE FROM OCS. A positive association has been observed between amount of estrogen and progestogen in OCs and vascular disease risk. A decline in serum high density lipoproteins (HDL) is reported with many progestational agents. Serum HDL decline is associated with increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, net effect depends on balance achieved between doese of estrogen and progestogen and nature and absolute amount of progestogen used. Consider amount of both hormones in the choice of an OC.

The dosage regimen prescribed should contain the least amount of estrogen and progestogen compatible with a low failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of

estrogen.

PERSISTENCE OF RISK OF VASCULAR DISEASE. Two studies have shown persistence of vascular disease risk for ever-users of OCs. In a U.S. study. MI risk after OC discontinuation persists for at least 9 years in women 40-49 years who had used OCs for five or more years; increased risk was not demonstrated in other age groups. In a study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after OCs stopped, although excess risk was very small. Both studies used OC formulations with 50 micrograms or higher of estropers.

scoped, almosping access risk was very similar both studies development of estrogens.

2. Estimates of Mortality from Contraceptive Use — A study using data from several sources concluded that with the exception of OC users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The possibility of increased mortality risk with age for OC users is based on data from the 1970s — but reported in 1983. However, current practice involves use of lower estrogen dose formulations combined with careful restriction of OC use to women without the various risk factors listed in this labeling.

Changes in practice and new data suggesting that cardiovascular disease risk with OCs may be less than previously observed prompted the Fertility and Maternal Health Drugs Advisory Committee to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with OC use after age 40 in healthy nonsmokers (even with newer low-dose formulations) greater potential health risks are associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if effective, acceptable contraception is not available.

The Committee concluded that the benefits of OC use by healthy nonsmoking women over 40 may outweigh the possible risks. Older women, as all women who take OCs, should use the lowest possible effective dose formulation.

3. Carcinoma of the Reproductive Organs — Numerous epidemiological studies have looked at the incidence of

formulation.

3. Carcimona of the Reproductive Organs — Numerous epidemiological studies have looked at the incidence of breast, endometrial, ovarian and centical cancer in women using OCs. Overwhelming evidence suggests that CC use is not associated with an increase in risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer, risk for at least a decade following long-term use. A few studies show a slightly increased relative risk of developing breast cancer, although the methodology of these studies, including differences in examination of users and nonusers, and in age at start of use, has been questioned.

Some studies suggest that CC use is associated with an increased risk of cervical intraepithelial neoplasia in some populations of women. However, controversy continues about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between OC use and breast and cervical cancers, a cause and effect relationship has not been established.

4. Hepatic Neoplasia — Benign hepatic adenomas are associated with OC use, although incidence is rare in the U.S. Indirect calculations estimate attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

British studies have shown an increased risk of hepatocellular carcinoma in long-term (> 8 years) OC users; these cancers are extremely rare in the U.S. and attributable risk (excess incidence) of liver cancers in OC users approaches less than one per million users.

5. Ocular Lesions — There are clinical case reports of retinal thrombosis with OC use. Discontinue OCs if there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions; undertake appropriate diagnostic and therapeutic measures immediately.

lesions; undertake appropriate diagnostic and therapeutic measures immediately, 6. Oral-Contracepture Use Betine or During Farty Prognancy.— Extensive epidemiological studies revealed no increased risk of birth defects when OCs used prior to pregnancy Studies do not suggest a teratogenic effect, particularly insodar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. OC-induced withdrawal bleeding should not be used as a pregnancy test. Do not use OCs during pregnancy to treat threatened or habitual abortion. Rule out pregnancy if two consecutive periods missed before continuing OC use. If oatlent has not adhered to prescribed schedule, consider pregnancy at time of first missed period. Discontinue OC if pregnancy confirmed.

7. Galibladder Disease—Earlier studies reported an increased lifetime relative risk of galibladder surgery in users of OCs and estrogens; more recent studies show that the relative risk of developing galibladder disease among OC users may be minimal, which may be related to use of formulations with lower hormonal estrogen and propestogen doses.

8. Carbohydrate and Lipid Metabolic Effects — OCs cause glucose intolerance in a significant percentage of users OCs with greater than 75 µg of estropen cause hyperinsulinism; lower estropen doses cause less glucose intolerance. Progestopens increase insulin secretion and create insulin resistance (effect varies with different agents). Observe prediabetic and diabetic women carefully while taking OCs. In non-diabetic women, OCs have no apparent effect on fasting blood glucose.

no application effect on inasting sound spicoses.

A small proportion of women will have persistent hypertriglyceridemia while on OCs. Changes in serum triglycerides and lipoprotein levels have been reported in OC users (see Warnings).

9. Elevated Blood Pressure—Increase in blood pressure has been reported in women on OCs, increase is more likely in older OC users and with continued use. Data show that incidence of hypertension increases with

likely in older OC users and with continued use. Data show that incidence of hypertension increases with increasing quantities of progestogen of progestogen and increasing quantities of progestogen of hypertension or hypertension-related diseases, or renal disease to use another contraceptive method. Monitor hypertensive women electing to use OCs closely, discontinue OC if significant blood pressure elevation occurs For most women, elevated blood pressure returns to normal after OC stopped. No difference in occurrence of hypertension among ever- and never-users exists.

10. Headache — Discontinue OC and evaluate cause at onset or exacerbation of higraine, or if new pattern of headache (i.e. recurrent, persistent, severe) develops.

11. Bleeding irregularities — Breakthrough bleeding and spotting sometimes occur, especially during first 3 months of use. Type and dose of progestogen may be important. Consider non-hormonal causes and take adequate diagnostic measures to rule out malignancy or pregnancy in event of breakthrough bleeding, as with any abnormal vaginal bleeding. If pathology excluded, time or a formulation change may solve the problem. In the event of amenorrhea, rule out pregnancy. Some women encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

event of amenormae, rule out pregnancy. Some women encounter post-pin amenormea or oligomenormea, especially when such a condition was pre-existent.

Procautions

1. Physical Examination and Follow Up — A complete medical history and physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent ahommal vaginal bleeding, conduct appropriate diagnostic measures to rule out malignancy. Monitor women with strong tamily history of breast cancer or who have breast nodules with particular care. 2. Lipid Disorders — Follow women being treated for hyperlipidemias closely if they elect to use OCs. Some progestogens may elevate LDL levels and may render control of hyperlipidemias more difficult. (See Warnings): 3. Liver Function — Discontinue OC if jaundice develops Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. Fluid Retention — OCs may cause some degree of fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions possibly aggravated by fluid retention. 5. Emotional Disorders — If significant depression occurs stop medication and use alternate contraceptive method in attempts to determine if symptom is drug related. Observe carefully those with history of degression and stop drug if depression recurs to serious degree. 6. Contact

Lenses — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities are associated with concomitant rifampin use A similar association, though less marked, is suggested with barbiturates, henryblutazone, phenytoin sodium, and possibly with prisorlytivin. and menstrual irregularities are associated with concomitant rifampin use. A similar association, though less marked, is suggested with barbiturates, phenylbufazone, phenylon sodium, and possibly with griseofulvin, ampicillin and tetracyclines. B. Interactors with Laboratory Tests—Certain endocrine—and liver-function tests and blood components may be affected by OCs: a. Increased prothrombin and factors VII. VIII. IX, and X, decreased antithrombin 3: increased norepinephrine-induced platelet aggregability b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI). 14 by column or by radioimmunoassay Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. C. Other binding proteins may be elevated in serum d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids. Tree or biologically active levels remain unchanged. e. friglycerides may be increased f. Glucose tolerance may be decreased g. Serum folate levels may be depreased by OCs. This may be of clinical significance if woman becomes pregnant shortly after stopping OC. 9. Carcinogenesis — See Warnings section. 10. Pregnancy — Pregnancy Category X. See Contraindications and Warnings. 11. Nursing Mothers — Small amounts of OC steroids have been identified in milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, OCs given in postpartum period may interfere with lactation by decreasing breast milk cunnity and quality If possible, advise nursing mother to use other forms of contraception, not OCs. until child is completely wearsed. completely weaped

compilerly wearned.

Information for the Patient — See Patient Package Labelling.

Adverse Reactions — An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis: arterial thromboembolism; pulmonary embolism; myocardial infarction cerebral hemorrhage; cerebral thrombosis; hypertension; galloladder disease; nepatic adenomas or benign liver turnors

cerebral hemorrhagic cerebral thrombosis; hypertension; gallbladder disease; hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and OC use, although additional confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis. The following adverse reactions have been reported in patients on OCs and are believed to be drug-related; nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorhea; temporary intertility; after treatment discontinued; edema; melasma which may persist; breast changes; tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical etosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash lallergid; mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in OC users and the association is neither confirmed nor refuted; congenital anomalies; premenstrual syndrome; cataracts; ontic neuritis; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsuitism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria; impaired renal function, hemolytic uremic syndrome; Budd-Chiari syndrome; acne; changes in iibidd; colinis; sickle-cell disease; cerebral-vascular disease with mitral valve prolages; lupus-like syndromes.

Deverdosage—Serious iil effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health** Benefits — The following noncontraceptive health benefits related to OC use are supported by epidemiological studies that largely utilized OC formulations containing doses exceeding 0.035

decreased incidence of ovarian cancer.

Dosage and Administration — For maximum contraceptive effectiveness, take TRIPHASIL* (levonorgestrel and ethinyl estraciol tablets — triphasic regimen 21- and 28-day regimens) exactly as directed and at intervals not over 24 hours.

(If TRIPHASIL* is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.) For full details on dosage and administration see prescribing information in package insert.



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The Editors and Mosby-Year Book, Inc., take this opportunity to acknowledge those reviewers who have contributed many hours and much effort in the evaluation of manuscripts submitted to the JOURNAL during the past year. Without their interest and help, publication of the JOURNAL would be very difficult indeed. We also continue to seek advice from reviewers and readers alike as regards their overall evaluation of the JOURNAL.

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Baird, David, Edinburgh, Scotland Baker, Carol J., Houston, Texas Baker, David A., Stony Brook, New York Baker, Elizabeth, Columbia, South Carolina Baker, Victoria V., Cincinnati, Ohio Ball, Harrison G., Boston, Massachusetts Ballard, Charles A., Los Angeles, California Balmaceda, Jose P., Laguna Hills, California Bamforth, Stephen J., Edmonton, Alberta, Canada Banta, David, The Hague, Netherlands Bantle, John P., Minneapolis, Minnesota Baram, David, Rochester, New York Barber, Hugh R.K., New York, New York Barbieri, Robert L., Stony Brook, New York Barclay, David L., Little Rock, Arkansas Barclay, Mel, Ann Arbor, Michigan Bard, David S., Little Rock, Arkansas Barden, Tom P., Cincinnati, Ohio Barker, Steven, Orange, California Barnes, Randall B., Chicago, Illinois Barr, Ronald J., Irvine, California Barrett, Rolland J., Winston-Salem, North Carolina Barron, William, Chicago, Illinois Barss, Vanessa, Boston, Massachusetts Bartholomew, Deborah A., Biloxi, Mississippi Bartlik, Barbara, New York, New York Bartolucci, Louis, San Francisco, California Bartscht, Karen, Ann Arbor, Michigan Bast, Robert C., Durham, North Carolina Bates, George William, Greenville, South Carolina Bates, James, Danville, Pennsylvania Battaglia, Frederick, Denver, Colorado Bawdon, Roger, Dallas, Texas Baxi, Laxmi, New York, New York Bayer, Steven, Boston, Massachusetts Baylis, Chris, Morgantown, West Virginia Bazer, F.W., Gainesville, Florida Beall, Marie H., Los Angeles, California Beard, Richard W., London, England Beck, Aaron T., Philadelphia, Pennsylvania Beck, R. Peter, Richmond, Virginia Becktell, Phoebe, Albuquerque, New Mexico Beer, Alan E., North Chicago, Illinois Behrman, Harold R., New Haven, Connecticut Beiser, Morton, Vancouver, British Columbia, Canada Belinson, Jerome L., Cleveland, Ohio Bell-Unger, Kathleen, San Francisco, California Benacerraf, Beryl, Boston, Massachusetts Benedetti, Thomas J., Seattle, Washington Benigno, Benedict B., Atlanta, Georgia Benirschke, Kurt, La Jolla, California Bennett, Bill, Jackson, Mississippi Benrubi, Guy, Jacksonville, Florida Benson, J. Thomas, Indianapolis, Indiana Benson, Ralph C., Portland, Oregon Bent, Alfred E., Long Beach, California Berchuck, Andrew, Durham, North Carolina Berek, Jonathan S., Los Angeles, California Berg, Barbara, Lexington, Kentucky Berger, Charlene, Westmount, Quebec, Canada

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Clapp, James Ford, III, Cleveland, Ohio Clark, David A., Hamilton, Ontario, Canada Clark, Kenneth E., Cincinnati, Ohio Clark, Robin, Loma Linda, California Clark, Steven Leigh, Provo, Utah Clarke, G.N., Carlton, Victoria, Australia Clarke-Pearson, Daniel L., Durham, North Carolina Cleary, Paul, Boston, Massachusetts Cleary, Robert E., Indianapolis, Indiana Clemmons, David R., Chapel Hill, North Carolina Clemons, Gisela K., Berkeley, California Coddington, Charles C., Norfolk, Virginia Cogan, Rosemary, Lubbock, Texas Cohen, Arnold W., Philadelphia, Pennsylvania Cohen, Carmel J., New York, New York Cohen, Jacques, New York, New York Cohen, Jesse M., West Orange, New Jersey Cohen, Leeber, Chicago, Illinois Cohen, Richard, Pittsburgh, Pennsylvania Cohen, Sheila, Stanford, California Cohen, Wayne R., Bronx, New York Collea, Joseph V., Washington, D.C. Collins, John C., Hamilton, Ontario, Canada Collins, John L., St. Louis, Missouri Colon, J.M., Newark, New Jersey Coltart, T.M., London, England Comstock, Christine H., Royal Oak, Michigan Coney, Pon Jola, Tucson, Arizona Connell, Elizabeth B., Atlanta, Georgia Conover, Wayne B., Lexington, Kentucky Cook, Christine, Louisville, Kentucky Copel, Joshua A., New Haven, Connecticut Copeland, Larry J., Columbus, Ohio Cordero, Leandro, Columbus, Ohio Corfman, Randel S., Rochester, Minnesota Corson, Stephen L., Philadelphia, Pennsylvania Cotton, David B., Detroit, Michigan Coulam, Carolyn B., Fairfax, Virginia Coury, Daniel, Columbus, Ohio Cousins, Larry, Loma Linda, California Coustan, Donald R., Providence, Rhode Island Coutifaris, Christos, Philadelphia, Pennsylvania Cowan, Bryan Dean, Jackson, Mississippi Cowchock, Susan, Philadelphia, Pennsylvania Cowett, Richard, Providence, Rhode Island Cox, Susan, Lexington, Kentucky Craig-Schmidt, Margaret, Auburn, Alabama Cramer, Daniel W., Boston, Massachusetts Crandall, Barbara F., Los Angeles, California Crane, James P., St. Louis, Missouri Crankshaw, Denis, London, Ontario, Canada Crawford, Michael, London, England Creasman, William T., Charleston, South Carolina Creasy, Robert K., Houston, Texas Crenshaw, Carlyle, Baltimore, Maryland Crinella, Francis M., Costa Mesa, California Crisp, William E., Phoenix, Arizona Cromblehome, William R., San Francisco, California Crosby, Warren M., Oklahoma City, Oklahoma Cruikshank, Dwight, Salt Lake City, Utah

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Fritz, Lin, Irvine, California Fu, Yao S., Los Angeles, California Fuchs, Anna-Riita, New York, New York Fuchs, Fritz, New York, New York Fuchs, P.G., Erlangen, Germany Fuller, Arlan, Boston, Massachusetts Futchner, Carlos, Santa Cruz, Bolivia Gabbe, Steven G., Columbus, Ohio Gabert, Harvey, New Orleans, Louisiana Gagnon, Robert F., London, Ontario, Canada Galask, Rudolph P., Iowa City, Iowa Galdenburg, Rabit, Birmingham, Alabama Gall, Stanley A., Louisville, Kentucky Galle, Phillip, Springfield, Illinois Gallion, Holly, Lexington, Kentucky Gallup, Donald G., Augusta, Georgia Gambone, Joseph Charles, Los Angeles, California Gambrell, R. Don, Augusta, Georgia Ganong, William F., San Francisco, California Gant, Norman F., Jr., Dallas, Texas Garcia, Celso-Ramon, Philadelphia, Pennsylvania Gardner, Vance O., Orange, California Garfield, Robert E., Hamilton, Ontario, Canada Garite, Thomas J., Orange, California Garratty, George, Los Angeles, California Gast, Michael J., St. Louis, Missouri Gates, Elana, San Francisco, California Gauthier, Philippe, Montreal, Quebec, Canada Gazzaniga, Alan B., Orange, California Geisler, Hans E., Indianapolis, Indiana Gerbie, Albert, Chicago, Illinois Gershenson, David M., Houston, Texas Gibb, William, Ottawa, Ontario, Canada Gibbs, Charles, Denver, Colorado Gibbs, Ronald S., Denver, Colorado Gibson, Mark, Burlington, Vermont Gifford, Ray, Cleveland, Ohio Gilbert, Raymond D., Loma Linda, California Giles, Harlan R., Pittsburgh, Pennsylvania Gilstrap, Larry C., III, Dallas, Texas Gindoff, Paul, Washington, D.C. Gingras, Jeannine L., Durham, North Carolina Ginsburg, Kenneth, Detroit, Michigan Girtanner, Robert E., Houston, Texas Gise, Leslie Hartley, New York, New York Gittelman, David K., Chapel Hill, North Carolina Giudice, Linda, Stanford, California Given, Fred T., Norfolk, Virginia Glinter, Kenneth P., Farmington Hill, Michigan Gluck, Louis, Orange, California Golan, Abraham, Zerifin, Israel Golbus, Mitchell S., San Francisco, California Goldberg, James D., San Francisco, California Goldberg, Jeff, Cleveland, Ohio Goldberg, Michael I., New Brunswick, New Jersey Golde, Steven H., Burbank, California Goldenberg, Robert L., Birmingham, Alabama Goldfarb, Alvin F., Philadelphia, Pennsylvania Goldkrand, John W., Savannah, Georgia Goldman, Janis V., Los Angeles, California

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Hammond, Mary G., Chapel Hill, North Carolina Hamparian, Vincent F., Columbus, Ohio Hampton, Harriette, Jackson, Mississippi Hancock, Kenneth C., Lackland, Texas Haney, Arthur F., Durham, North Carolina Hanjani, Parviz, Gladwyne, Pennsylvania Hankins, Gary V., Lackland, Texas Hannigan, Edward V., Galveston, Texas Hanson, Frederick W., Sacramento, California Hapidou, Eleni G., London, Ontario, Canada Harbert, Guy M., Jr., Charlottesville, Virginia Harbon, Simone, Orsay, France Harger, James, Pittsburgh, Pennsylvania Hargrove, Joel T., Columbia, Tennessee Harman, Christopher, Winnipeg, Manitoba, Canada Harper, Margaret A., Winston-Salem, North Carolina Harper, Michael, San Antonio, Texas Harrigan, John, New Brunswick, New Jersey Harris, Tamara, Hyattsville, Maryland Harrison, Michelle, Pittsburgh, Pennsylvania Harrison, Victoria, Baltimore, Maryland Harrison-Hohner, Jane A., Portland, Oregon Hartlage, Shirley, Chicago, Illinois Haseltine, Florence P., Bethesda, Maryland Hatch, Kenneth D., Tucson, Arizona Hatjis, Christos G., Columbus, Ohio Hauth, John C., Birmingham, Alabama Hayashi, Robert H., Ann Arbor, Michigan Hayashi, Terry T., Pittsburgh, Pennsylvania Hayden, Glenn E., Seattle, Washington Hayes, Patricia M., Richmond, Virginia Hayes, Peggy, Atlanta, Georgia Hayes, Rosalind A., Columbus, Ohio Hays, James M., Knoxville, Tennessee Hayslett, John P., New Haven, Connecticut Hazzard, William R., Winston-Salem, North Carolina Head, Roxane, San Francisco, California Healy, David L., Clayton, Australia Heber, Ruth R., New York, New York Hebert, Lee, Columbus, Ohio Hegge, Frederick, Portland, Oregon Heinrichs, W. LeRoy, Stanford, California Heller, Debra S., New York, New York Heller, Paul B., Philadelphia, Pennsylvania Hemsell, David L., Dallas, Texas Henderson, Perry A., Madison, Wisconsin Henderson, Simon R., San Francisco, California Hendricks, Charles, Chapel Hill, North Carolina Hendricks, Susan K., Seattle, Washington Henrik, Elizabeth, West Montreal, Quebec, Canada Henry, George, Denver, Colorado Hensleigh, Paul A., San Jose, California Herbert, Sarah E., Atlanta, Georgia Herbold, David R., Wichita, Kansas Hernandez, Eleuterio R., Baltimore, Maryland Hernandez, Enrique, Philadelphia, Pennsylvania Herz, Elisabeth K., Washington, D.C. Hess, Allan D., Baltimore, Maryland Hess, Carl, Orange, California Hess, L. Wayne, Jackson, Mississippi

Hickock, Durlin E., Seattle, Washington Hicks, Dorothy Jane, Miami, Florida Hickson, Rcbert, Chicago, Illinois Higgin, John R., Calgary, Alberta, Canada Hilgers, Robert D., Springfield, Illinois Hill, Gale, Durham, North Carolina Hill, George L., Nashville, Tennessee Hill, Joseph A., III, Boston, Massachusetts Hill, Lyndon M., Pittsburgh, Pennsylvania Hill, Washington C., Nashville, Tennessee Hillard, Paula J. Adams, Cincinnati, Ohio Hirsch, Hans A., Tuebingen, Germany Hirsch, Lisa B., Orange, California Hobbins, John C., New Haven, Connecticut Hobel, Calvin J., Los Angeles, California Hodgen, Gary D., Norfolk, Virginia Hofmann, Glen E., New York, New York Hogan, W. Michael, Wynnewood, Pennsylvania Hogge, Allen, Baltimore, Maryland Hollenbach, Kathryn A., Los Angeles, California Hollingsworth, Claude, Charlotte, North Carolina Hollingsworth, Dorothy, San Diego, California Holmes, K_ng Kennard, Seattle, Washington Holtz, Gar 1, Mount Pleasant, South Carolina Holzgreve, Wolfgang, Muenster, Germany Holzman, Gerlad, Augusta, Georgia Homer, Charles J., Boston, Massachusetts Homesley, Howard D., Winston-Salem, North Carolina Homm, Robert J., Lexington, Kentucky Hoppenbrouwers, Toke, Los Angeles, California Horbach, Nicolette S., Washington, D.C. Horger, Edgar O., III, Charleston, South Carolina Hornstra, Gerald, Maastricht, Netherlands Horrobin, David F., Kentville, Nova Scotia, Canada Hoskins, William J., New York, New York Hosn, Kambiz, Orange, California Howie, P. N., Dundee, Scotland Hoyme, Udo B., Essen, Germany Hreshchyshyn, Myroslaw M., Buffalo, New York Huch, Renate, Zurich, Switzerland Huddleston, John F., Atlanta, Georgia Huff, Robert W., San Antonio, Texas Hufnagel Vicki, Los Angeles, California Hughes, Claude, Durham, North Carolina Hughes, David A., London, England Hughes, J.R.V., London, England Hughey, Michael, Chicago, Illinois Huhta, James C., Philadelphia, Pennsylvania Hulka, Barbara, Chapel Hill, North Carolina Hulka, Jaroslav F., Chapel Hill, North Carolina Hull, Magdalen, Stony Brook, New York Hull, Water, Columbus, Ohio Hung, Terry T., Miami, Florida Hunt, Joan, Kansas City, Kansas Hunter, Richard E., Worcester, Massachusetts Hurt, Glen, Richmond, Virginia Husemezer, R.P., Grantham, England Husseinzadeh, Nader, Cincinnati, Ohio Huszar, Gabor B., New Haven, Connecticut Hwang, Daniel, Baton Rouge, Louisiana

Iams, Jay D., Columbus, Ohio Iffy, Leslie, Newark, New Jersey Ignarro, Louis, Los Angeles, California Ing, Todd, Hines, Illinois Isaacs, John H., Maywood, Illinois Ismail, Mahmoud, Chicago, Illinois Israel, Robert, Los Angeles, California Iversen, Ole-Erik, Sykeus, Norway Jackson, Rebecca, Columbus, Ohio Jacobs, Mark, San Francisco, California Jaffe, Robert B., San Francisco, California James, L. Stanley, New York, New York James, Mark E., Atlanta, Georgia Jarrell, Maureen A., Burlington, Vermont Jeanty, Phil, Nashville, Tennessee Jelovsek, Frederick R., Little Rock, Arkansas Jennings, Ralph, Jr., Lakeland, Florida Jensen, H.K., Aalborg, Denmark Jobe, A., Torrance, California Johnson, Gary, Salt Lake City, Utah Johnson, I.R., Nottingham, England Johnson, John Harvey, Dallas, Texas Johnson, John W.C., Gainesville, Florida Johnson, Michael L., Denver, Colorado Johnson, Robert, Atlanta, Georgia Johnson, Susan R., Iowa City, Iowa Johnson, Timothy R.B., Baltimore, Maryland Jones, Edward G., Irvine, California Jones, Georgia S., Norfolk, Virginia Jones, Howard W., III, Nashville, Tennessee Jones, Lovell A., Houston, Texas Jones, Robert B., Indianapolis, Indiana Josephs, Laura, New York, New York Josimovich, John B., Brooklyn, New York Jovanovic, Lois, Santa Barbara, California Judd, Howard L., Los Angeles, California Kacinski, Barry M., New Haven, Connecticut Kalinich, Lila J., New York, New York Kaminetzky, Harold A., Washington, D.C. Kao, Ming-Shian, St. Louis, Missouri Karlan, Beth Y., Los Angeles, California Karnitis, Joseph, Columbus, Ohio Karram, Michael, Cincinnati, Ohio Katz, Michael, San Francisco, California Katz, Vern, Chapel Hill, North Carolina Kaufman, Raymond H., Houston, Texas Kaufmann, Robert C., Springfield, Illinois Kauma, Scott W., Richmond, Virginia Kaunitz, Andrew M., Jacksonville, Florida Kauppila, Antti, Oulu, Finland Kawada, Charles, Boston, Massachusetts Kay, Helen, Durham, North Carolina Kazazian, Haig H., Baltimore, Maryland Keegan, Kirk A., Orange, California Kegeles, S. Stephen, Farmington, Connecticut Keith, Louis, Chicago, Illinois Kellogg, Spencer F., Miami, Florida Kelly, JoAnn, Pasadena, California Kelly, Randall T., Dearborn, Michigan Kelsey, Jennifer L., New York, New York

Kennard, Beth, Columbus, Ohio Kennison, Robert D., Boston, Massachusetts Kessel, Bruce, Cincinnati, Ohio Keye, William R., Salt Lake City, Utah Kiely, John, Hyattsville, Maryland Killam, Allen P., Durham, North Carolina Kim, Moon H., Columbus, Ohio King, Theodore, Baltimore, Maryland Kirk, E. Paul, Portland, Oregon Kirkley, William H., St. Lauderdale, Florida Kirschbaum, Thomas H., Bronx, New York Kirshon, Brian, Houston, Texas Kitchin, James D., III, Charlottesville, Virginia Kitterman, Joseph, San Francisco, California Kitzmiller, John L., San Francisco, California Kjos, Siri, Los Angeles, California Klaus, Hanna, Bethesda, Maryland Klein, Luella, Atlanta, Georgia Kleiner, George, Larchmont, New York Kleinman, Joel, Hyattsville, Maryland Kleitman, Nathaniel, Santa Monica, California Kleppinger, Richard K., West Reading, Pennsylvania Kletzky, Oscar A., Torrance, California Kliot, David A., Brooklyn, New York Kluzak, Thomas R., Wichita, Kansas Knapp, Robert C., Boston, Massachusetts Knaus, John V., Evanston, Illinois Kniss, Douglas, Columbus, Ohio Knox, Eric, Minneapolis, Minnesota Knuppel, Robert A., New Brunswick, New Jersey Kobayashi, Fuminori, Kobe, Japan Kochenour, Neil K., Salt Lake City, Utah Koff, Steven, Columbus, Ohio Kofinas, George D., Brooklyn, New York Kohn, Elise, Bethesda, Maryland Kohn, N.N., Willingboro, New Jersey Kohorn, Ernest I., New Haven, Connecticut Kolterman, Orville, San Diego, California Koob, Thomas J., Boston, Massachusetts Koos, Brian John, Los Angeles, California Koplik, Lewis H., Albuquerque, New Mexico Korn, G.W., Vancouver, British Columbia, Canada Kovacs, Bruce W., Los Angeles, California Krantz, Kermit E., Kansas City, Kansas Kraus, Frederick T., St. Louis, Missouri Kraybill, Ernest, Chapel Hill, North Carolina Krebs, H.B., Anandale, Virginia Krener, Penelope, Davis, California Kreutner, A. Karen, Charleston, South Carolina Krumholz, Burton, New Hyde Park, New York Kuhlman, Kathleen Ann, Philadelphia, Pennsylvania Kuhnert, Betty R., North Wales, Pennsylvania Kurman, Robert J., Baltimore, Maryland Kurtz, Alfred B., Philadelphia, Pennsylvania Kush, Michael J., DuBois, Pennsylvania Kute, Timothy E., Winston-Salem, North Carolina Kutzner, Susan, Houston, Texas LaBarbera, Andrew R., Cincinnati, Ohio LaFerla, John J., St. Paul, Minnesota LaPolla, James, Tampa, Florida

Laatikainen, Timo, Oulu, Finland Labrum, Anthony H., Rochester, New York Lacey, Conley G., La Jolla, California Lafond, J., Montreal, Quebec, Canada Lagrew, David C., Laguna Hills, California Lalinec-Michaud, Martine, Verdun, Quebec, Canada Lamb, Emmet J., Stanford, California Lamm, L., Aarhus, Denmark Lamont, J., Hamilton, Ontario, Canada Lancaster, Wayne, Detroit, Michigan Landers, Daniel, San Francisco, California Landon, Mark B., Columbus, Ohio Landy, Helaine, Washington, D.C. Langer, Oded, San Antonio, Texas Langton, P.D., Leicester, England Lansman, Henry, Miami, Florida Lanzendorf, Susan E., Beaverton, Oregon Lapolla, James, Temple Terrace, Florida Laros, Russell K., San Francisco, California Larrieux, J. Robert, Boston, Massachusetts Larsen, Bryan, Huntington, West Virginia Larsen, John W., Washington, D.C. Larson, Bryan, Huntington, West Virginia Larson, James, Newark, Delaware Lasley, William, Sacramento, California Laube, Douglas W., Iowa City, Iowa Laudenbach, Bonnie, Fitchburg, Massachusetts Lauersen, Niels, New York, New York Lauritzen, Ch., Ulm, Austria Lavin, Justin, Akron, Ohio LeClair, Gary J., Eugene, Oregon Lebherz, Thomas B.K., Los Angeles, California Lederman, Regina P., Galveston, Texas Ledger, William J., New York, New York Lee, N.C., Atlanta, Georgia Lee, Raymond A., Rochester, Minnesota Lee, Roger B., Tacoma, Washington Lee, Wesley, Royal Oak, Michigan Leech, R.W., Oklahoma City, Oklahoma Leiblum, Sandra R., Piscataway, New Jersey Lenke, Roger, Denver, Colorado Leonetti, Helene B., Bethlehem, Pennsylvania Leppert, Phyllis C., Rochester, New York Lessing, Joseph B., Tel Aviv, Israel Leung, Benjamin, Minneapolis, Minnesota Leveno, Kenneth J., Dallas, Texas Leventhal, John Mishel, New Haven, Connecticut Levinson, Carl, San Francisco, California Lewandowski, George, Columbus, Ohio Lewis, David F., Orange, California Lewis, George C., Philadelphia, Pennsylvania Lewis, John L., New York, New York Lewis, Steven H., Vero Beach, Florida Liao, Shu Yuan, Orange, California Lickrish, Gordon M., Toronto, Ontario, Canada Lieberman, Ellice, Boston, Massachusetts Liedholm, Percy, Malmø, Sweden Liggins, G.D., Auckland, New Zealand Lilford, Richard, Leeds, England Lim, David, Columbus, Ohio

Lin, Fritz, Orange, California Lin, James, Cincinnati, Ohio Lin, Y.C., Columbus, Ohio Lindheimer, Marshall D., Chicago, Illinois Lindsay, Michael K., Atlanta, Georgia Lindsay, R., Rochester, Minnesota Ling, Frank W., Memphis, Tennessee Lipshitz, Jeffrey, Las Vegas, Nevada Little, A. Brian, Montreal, Quebec, Canada Liu, James H., Cincinnati, Ohio Lobo, Rogerio A., Los Angeles, California Lockwood, Charles J., New York, New York Long, Cecil, Jackson, Mississippi Longo, Lawrence D., Loma Linda, California Lorenz, Robert P., Royal Oaks, Michigan Lossick, Joseph G., Atlanta, Georgia Lotgering, Frederick, Rotterdam, Netherlands Loucks, Anne, Athens, Ohio Low, James A, Kingston, Ontario, Canada Lowden, Robert J., Seattle, Washington Lowe, Nancy K., Columbus, Ohio Lowensohn, Richard, Portland, Oregon Lubbe, W.F., Auckland, New Zealand Lubs, Herbert, Miami, Florida Lucas, Joel, Columbus, Ohio Lucas, Michael, Dallas, Texas Luciano, Anthony A., Farmington, Connecticut Ludmir, Jack, Philadelphia, Pennsylvania Luna-Sotura, Armando, Houston, Texas Lupo, Virginia R., Minneapolis, Minnesota Lurain, John Robert, Chicago, Illinois Luthy, David A., Seattle, Washington Lutz, Dennis, Minot, North Dakota Lynch, Henry T., Omaha, Nebraska Lynch, Lauren, New York, New York Lyttle, Richard, Philadelphia, Pennsylvania Mabie, William, Memphis, Tennessee MacDonald, Paul C., Dallas, Texas MacVicar, J., Leicester, England Macgregor, Alexander H., Toledo, Ohio Machlin, Lawrence, Nutley, New Jersey Macri, Cynthia, Duarte, California Magness, Ronald R., Dallas, Texas Magos, A.L., Headington, Oxford, England Mahan, Charles S., Gainesville, Florida Mahoney, Maurice J., New Haven, Connecticut Main, Denise M., San Francisco, California Main, Elliott, San Francisco, California Major, Carol A., Orange, California Major, Francis J., Denver, Colorado Makowski, Edgar L., Denver, Colorado Malfetano, John H., Albany, New York Malinak, Lewis Russell, Houston, Texas Malone, John M., Jr., Detroit, Michigan Maltzer, Mark, Roseville, California Manetta, Alberto, Orange, California Mann, Leon I., Cleveland, Ohio Mann, William, Stony Brook, New York Mannel, Robert, Oklahoma City, Oklahoma Manning, Frank A., Winnipeg, Manitoba, Canada Marchant, Douglas J., Boston, Massachusetts Marcus, Robert, Palo Alto, California Margolin, Malcolm L., Beverly Hills, California Mari, Giancarlo, New Haven, Connecticut Marier, John, Ottawa, Ontario, Canada Mark, Carl, Portland, Oregon Marks, Melvin, Long Beach, California Marrs, Richard P., Los Angeles, California Marsal, Karel, Malmo, Sweden Marsh, Frank H., Denver, Colorado Marshall, Jean, Providence, Rhode Island Marshall, John, Torrance, California Marshall, John C., Ann Arbor, Michigan Martens, Mark G., Galveston, Texas Martimbeau, Pierre, Charlotte, North Carolina Martin, Chester B., Jr., Madison, Wisconsin Martin, Dan, Memphis, Tennessee Martin, Daniel Clyde, Memphis, Tennessee Martin, James N., Jackson, Mississippi Martin, John, St. Louis, Missouri Martin, Mary, San Francisco, California Martin, Rick, Jackson, Mississippi Martinez-Maza, Otoniel, Los Angeles, California Mathur, Subbi, Charleston, South Carolina Matt, Dennis W., Richmond, Virginia Mattingly, Stephen J., San Antonio, Texas Mattison, Donald R., Pittsburgh, Pennsylvania Mattox, John, Phoenix, Arizona Maulik, Dev, Prairie Village, Kansas Maxson, Wayne S., Margate, Florida Mayer, Kenneth, Pawtucket, Rhode Island McLaughlin, Margaret, Cincinnati, Ohio McCausland, Arthur M., Sacramento, California McConnachie, Peter R., Springfield, Illinois McCormack, William, Brooklyn, New York McCormick, Richard, South Bend, Indiana McDonald, Alison, Montreal, Quebec, Canada McDonald, John A., Columbus, Ohio McDonough, Paul G., Augusta, Georgia McGahan, John P., Sacramento, California McGaugh, James L., Irvine, California McGowan, Larry, Potomac, Maryland McGregor, James A., Denver, Colorado McGuire, Edward J., Ann Arbor, Michigan McGuire, William L., San Antonio, Texas McIntyre, John A., Indianapolis, Indiana McNeeley, S. Gene, Ann Arbor, Michigan McPherson, Dave L., Orange, California McRae, Mary Ann, Springfield, Illinois Mead, Philip Bartlett, Burlington, Vermont Meadows, Anna, Philadelphia, Pennsylvania Medearis, Arnold L., Rancho Palos Verdes, California Meeks, Rodney, Jackson, Mississippi Meier, Frederick A., Richmond, Virginia Meis, Paul J., Winston-Salem, North Carolina Meldrum, David R., Rancho Palos, California Mennuti, Michael T., Philadelphia, Pennsylvania Menon, K.M.J., Ann Arbor, Michigan Mercer, Lane, Chicago, Illinois Merenstein, Gerald B., Denver, Colorado

Merkatz, Irwin R., Bronx, New York Merkatz, Ruth, Bronx, New York Merril, James A., Seattle, Washington Merritt, Christopher, New Orleans, Louisiana Mersol-Barg, Michael, Grosse Point Farm, Michigan Meschia, Giacomo, Denver, Colorado Mestman, Jorge, Los Angeles, California Metzger, Boyd E., Chicago, Illinois Metzger, Debbie, Farmington, Connecticut Meyer, Marjorie C., Burlington, Vermont Meyers, Mary E., Encinitas, California Meyskens, Frank L., Orange, California Michaelson, Karen, Cheney, Washington Mikesell, Susan G., Washington, D.C. Miller, David Scott, Chicago, Illinois Miller, Frank C., Lexington, Kentucky Miller, John Preston, San Francisco, California Miller, Joseph M., New Orleans, Louisiana Miller, Kurt, Madison, Wisconsin Miller, Langdon L., Bethesda, Maryland Miller, R. David, Tustin, California Mills, James, Bethesda, Maryland Milton, Lee, Los Angeles, California Milunsky, Aubrey, Boston, Massachusetts Minkler, Donald H., Berkeley, California Minkoff, Howard, Brooklyn, New York Miodovnik, Menachem, Cincinnati, Ohio Mironneau, Jean, Paris, France Mishell, Daniel R., Los Angeles, California Misri, Shaila, Vancouver, British Columbia, Canada Mitchell, Bryan F., Edmonton, Alberta, Canada Mitchell, Murray D., Salt Lake City, Utah Miyazawa, Kunio, Bethesda, Maryland Moawad, Atef H., Chicago, Illinois Modanlou, Houchang D., Long Beach, California Moessinger, Adrien, Berne, Switzerland Moffett, Alfred H., Jr., Leesburg, Florida Moghissi, Kamran S., Detroit, Michigan Moise, Kenneth J., Jr., Houston, Texas Moll, Waldemar, Regensburg, Germany Moller, Birger R., Odense, Denmark Molsted-Pedersen, Lars, Copenhagen, Denmark Monif, Gilles R.G., Omaha, Nebraska Montag, Thomas W., Burlingame, California Montz, F.J., Los Angeles, California Moore, H. Christopher, Orange, California Moore, Jerry G., Sylmar, California Moore, Thomas R., San Diego, California Morales, Walter, Orlando, Florida Morgan, Mark, Oklahoma City, Oklahoma Morley, George W., Ann Arbor, Michigan Morretti, Michael, Memphis, Tennessee Morrison, John C., Jackson, Mississippi Morrow, Charles P., Los Angeles, California Morrow, Paul, Los Angeles, California Mortel, Rodrigue, Hershey, Pennsylvania Mortola, Joseph, Boston, Massachusetts Mostel, Arthur P., Stamford, Connecticut Moulton, Bruce, Cincinnati, Ohio Moutquin, Jean, Quebec City, Quebec, Canada

Moya, Fernando, Dallas, Texas Muasher, S.J., Norfolk, Virginia Mueller, Charles, Columbus, Ohio Mueller-Heubach, Eberhard, Winston-Salem, North Carolina Mulchahey, Kristi M., Birmingham, Alabama Muller, P., Basel, Switzerland Muram, David, Memphis, Tennessee Murata, Yuji, Orange, California Muse, Kenneth, Lexington, Kentucky Mutch, David Gardner, St. Louis, Missouri Myatt, Leslie, Cincinnati, Ohio Myazaki, F., Los Angeles, California Myers, Adam K., Washington, D.C. Nachtigall, Lila, New York, New York Nachtigall, Robert, San Francisco, California Nadelson, Carol, Boston, Massachusetts Naeye, Richard L., Hershey, Pennsylvania Naftolin, Frederick, New Haven, Connecticut Nagamani, Manubai, Galveston, Texas Nageotte, Michael P., Long Beach, California Nagey, David A., Baltimore, Maryland Nagourney, Robert, Irvine, California Nakajima, Steven T., Burlington, Vermont Nanninga, John B., Chicago, Illinois Nasrallah, Henry, Columbus, Ohio Nathanielsz, Peter W., Ithaca, New York Naulty, Stephen, Washington, D.C. Navar, Gabriel L., New Orleans, Louisiana Neff, John, Columbus, Ohio Neff, Norton, Columbus, Ohio Nelson, Donald Michael, St. Louis, Missouri Nelson, Karin B., Bethesda, Maryland Nelson, Lewis H., Winston-Salem, North Carolina Nestler, John E., Richmond, Virginia Neuwalder, Herbert F., Tenafly, New Jersey Neuwirth, Robert S., New York, New York Newton, Edward R., San Antonio, Texas Newton, John R., Birmingham, England Ney-Helmke, Denise, Madison, Wisconsin Nichols, David H., Providence, Rhode Island Nicolaides, Kypros H., London, England Niebyl, Jennifer R., Iowa City, Iowa Niloff, Jonathan M., Boston, Massachusetts Nimrod, Carl, Calgary, Alberta, Canada Nisce, Lourdes Z., New York, New York Nolan, Thomas E., Augusta, Georgia Noller, Kenneth L., Worcester, Massachusetts Norstedt, Gunnar, Huddinge, Sweden Notelovitz, Morris, Gainesville, Florida Notman, Malkah T., Brookline, Massachusetts Noumoff, Joel S., Philadelphia, Pennsylvania Novy, Miles J., Portland, Oregon Nugent, Clark E., Ann Arbor, Michigan Nunez, Margaret Miller, Charlotte, North Carolina Nunley, Wallace C., Jr., Charlotte, North Carolina Nuovo, Gerard, Stony Brook, New York Nusbaum, Geoffrey D., Haddonfield, New Jersey Nuwayhid, Bahij, Montreal, Quebec, Canada Nyberg, David A., Seattle, Washington

O'Brien, Timothy J., Little Rock, Arkansas O'Brien, William F., Tampa, Florida O'Connor, Dennis M., Bethesda, Maryland O'Connor, John F., New York, New York O'Dorissio, Tom, Columbus, Ohio O'Hara, Michael W., Iowa City, Iowa O'Leary, James A., Bethlehem, Pennsylvania O'Quinn, April, New Orleans, Louisiana O'Shaughnessy, Richard W., Columbus, Ohio O'Sullivan, Mary Jo, Miami, Florida O'Toole, Robert, Columbus, Ohio Odell, W.D., Salt Lake City, Utah Ogburn, Paul, Rochester, Minnesota Oh, William, Providence, Rhode Island Oi, Richard H., Sacramento, California Olive, David L., San Antonio, Texas Olson, David M., London, Ontario, Canada Orr, James W., Lakeland, Florida Orsini, Margaret, Madison, Wisconsin Ory, Steven J., Rochester, Minnesota Osborne, Newton G., Syracuse, New York Osei, Kwame, Columbus, Ohio Oski, Frank, Baltimore, Maryland Ostergard, Donald R., Long Beach, California Ott, William J., St. Louis, Missouri Overstreet, James W., Davis, California Owman, Christer, Bethesda, Maryland Paddison, Patricia, Brooklyn, New York Page, Leslie, Fort Scott, Kansas Palmer, Sue Mary, Houston, Texas Pardi, Giorgio, Milan, Italy Parer, Julian T., San Francisco, California Parham, Groesbeck P., Los Angeles, California Pariser, Steve, Columbus, Ohio Parisi, Valerie M., Houston, Texas Park, Robert C., Washington, D.C. Parker, C. Richard, Jr., Birmingham, Alabama Parr, Margie B., Carbondale, Illinois Parsons, Anna, Tampa, Florida Pastorek, Joseph G., New Orleans, Louisiana Patsner, Bruce, Red Bank, New Jersey Pattillo, Roland A., Milwaukee, Wisconsin Paul, Michael, St. Louis, Missouri Paul, Richard H., Los Angeles, California Paulson, Richard J., Los Angeles, California Peaceman, Alan M., Houston, Texas Pearce, Patricia A., Halifax, Nova Scotia, Canada Pearce, William J., Loma Linda, California Pecorelli, Sergio, Brescia, Italy Peng, Thomas C., Richmond, Virginia Pent, David, Phoenix, Arizona Pepe, Gerald, Norfolk, Virginia Pergament, Eugene, Chicago, Illinois Perkins, Richard P., Omaha, Nebraska Petersen, Janice L., Denver, Colorado Peterson, Christine M., Charlottesville, Virginia Peterson, Herbert B., Atlanta, Georgia Petrie, Roy H., St. Louis, Missouri Petty, William, Portland, Oregon Phelan, Jeffrey P., West Covina, California

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PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION

Elevated fetal plasma lactate produces polyhydramnios in the sheep

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In human fetuses with hemolytic diseases such as erythroblastosis fetalis, hydrops fetalis or polyhydramnios often develops. The mechanism(s) that produces these fluid imbalances is unknown, although lactate concentrations have been reported to be elevated in hydropic human fetuses with erythroblastosis. In this study we explored the role of lactate in producing fetal fluid imbalances. In seven near-term fetal sheep, we infused 5 mol/L sodium lactate at a rate of 10 mmol/hr for 3 days. Fetal plasma lactate rose by 6.0 ± 1.0 (mean ± SE) mmol/L above control. Fetal plasma osmolality and Na+ increased slightly, CI⁻ decreased, and bicarbonate rose in proportion to the CI⁻ decrease. Fetal renal lactate excretion was 1.1 ± 0.3 mmol/hr while Na+ excretion was 10.6 ± 1.9 mEg/hr. Fetal urine flow increased by 1.9 \pm 0.4 L/day and the urine remained hypotonic relative to fetal plasma throughout the infusion. Amniotic fluid lactate and Na+ rose during the infusion period and remained elevated during a 24-hour recovery period. Amniotic plus allantoic fluid volume at autopsy was 5.3 ± 0.8 L compared with a normal of 0.5 to 1.0 L. There was little evidence of fetal edema. In summary, a moderate sustained elevation in fetal plasma lactate concentration appears to be a powerful osmotic agent for fetal accumulation of fluid from the maternal compartment over a period of days. This may be the primary mechanism whereby hydrops fetalis or polyhydramnios develops in severely anemic human fetuses, (AM J OBSTET GYNECOL 1991;165:1595-1607.)

Key words: Hydrops fetalis, erythroblastosis fetalis, sheep, fluid dynamics, urinary output, edema, amniotic fluid volume

Hydrops fetalis (severe fetal edema) and polydramnios (excessive amniotic fluid volume) often occur concomitantly with hemolytic anemias, but the mechanism(s) that produces these two conditions is unknown. Recent studies of erythroblastotic human fetuses^{1,2} have reported an elevated fetal plasma lactate concentration correlated inversely with plasma hemoglobin concentration and oxygen content. The critical values for oxygen content (2 mmol/L), hemoglobin concentration (4 gm/dl), and oxygen delivery rate (14 ml/kg/min)³ necessary to maintain adequate fetal tissue oxygenation also have been determined. These data, along with studies of blood flow during severe hypoxia,⁴ suggest that as the fetus becomes severely anemic and oxygen delivery drops below the critical level, a redistribution

of blood flow occurs. Systemic tissues then become hypoxic and begin producing lactate. In humans, only those fetuses with plasma lactate elevated to three to five times normal (i.e., 3 to 10 mmol/L) were hydropic.¹ The importance of this observation is that it may provide a mechanism for the development of hydrops or polyhydramnios; that is, as lactate accumulates in the fetal plasma, it may act osmotically to draw fluid from the maternal to the fetal circulation. The potential for producing fetal fluid imbalance stems from the fact that each millimole per liter increase in lactate at fetal body temperature could generate approximately 20 mm Hg osmotic pressure. This could provide a substantial driving force for water accumulation by the fetus.

The purpose of this study was to determine the role of elevated fetal plasma lactate in the development of hydrops fetalis and polyhydramnios. To study this, we infused concentrated sodium lactate (pH, 5.0) intravascularly into near-term fetal sheep at a rate sufficient to increase the plasma lactate concentration to three to-five times normal, i.e., comparable to the levels seen in edematous, anemic human fetuses. The infusion was continued for 3 days, during which time the fetuses were monitored closely and examined daily by ultrasonography for the development of polyhydramnios or ascites. The hypothesis was that lactate would act os-

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CONTROL	5 M Na	RECOVERY		
Day 1	Day 2	Day 3	Day 4	Day 5
4pm 8am	4pm 8am	4pm 8am	4pm 8am	4pm 8am
1 2	3 4	5	6	7 8

Sample #

Fig. 1. Schematic representation of sampling regimen used in this study.

motically to draw fluid from the maternal to the fetal circulation, potentially resulting in fetal edema, ascites formation, and an increased amniotic fluid volume.

Material and methods

Surgery and postoperative care. Seven pregnant ewes (gestational age at surgery 124 ± 1 days, mean \pm SE) were used in these studies. All procedures and experiments decribed in this study are in accordance with and have been approved by the University of California at San Diego Animal Subjects Committee. The experiments are in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Food was withheld 24 hours before surgery. Atropine (1 mg) was administered 30 minutes before surgery (intramuscularly) to reduce respiratory and gastric secretions during anesthesia. 5 Anesthesia was induced with 1 gm thiamylal sodium (Biotal, Bio-Ceutic) given intravenously; the animal was intubated and anesthesia was maintained with 0.5% to 1.5% halothane in oxygen. Indwelling catheters were aseptically placed in one maternal femoral artery and vein. Maternal heart rate and arterial pressure were monitored continuously during surgery, and the ewe received 1 L of lactated Ringer's solution with 5% dextrose intravenously during surgery. One liter of warmed lactated Ringer's solution was administered intravenously to the ewe, and 500 ml of warmed normal saline solution was administered to the amniotic space 2 hours after surgery to replace any fluids that might have been lost during surgery.

The surgical procedure involved exposing the fetus, to the level of the groin, through a uterine incision. Fetal femoral catheters were inserted bilaterally to the level of the diaphragm in the descending aorta and inferior vena cava. To expose the bladder, a suprapubic

abdominal incision of the fetus was performed. The bladder was incised; a catheter was inserted and secured by a purse-string ligature, and the abdomen was closed. Two amniotic fluid catheters were sutured to the fetal skin. The fetal membranes and uterus were closed separately, with care being taken to ensure there were no leaks in the membranes or uterus. All catheters were exteriorized into a cloth pouch secured to the flank of the ewe and all incisions were closed.

Vascular catheters were flushed daily with heparin sodium (1000 U/ml) to maintain patency. Fetuses received 0.4 gm ticarcillin and 13.4 mg clavulanate (a β -lactamase inhibitor) daily by an amniotic catheter, and the ewes received 500,000 U penicillin and 625 mg streptomycin (intramuscularly) daily for the first 5 post-operative days.

Experimental protocol. Experiments began 5 days after surgery. The protocol, illustrated in Fig. 1, consisted of a 24-hour control period, a 72-hour infusion period, and a 24-hour recovery period. Sample times during the 5-day study are indicated on Fig. 1. This experimental protocol was the same as that used in our recent study in which 5 mol/L sodium chloride was infused into ovine fetuses.6 In this study an infusion of 5 mol/L sodium chloride lactate into a fetal vein at a rate of 10 mmol/hr (volume approximately 2 ml/hr) was initiated at 9 AM (1 hour after the second control sample). Initially, the lactic acid infusate was titrated to a pH of approximately 7.0 by adding sodium hydroxide pellets. Infusion of this resulted in severe alkalosis of the fetus; those fetuses are not included in the results of this study. Subsequently the infusate was titrated to a pH of approximately 5.0. Throughout the first day fetal arterial blood lactate was monitored and the infusion rate altered if necessary to achieve a plasma lactate level of 5 to 10 mmol/L, i.e., a level comparable to

Table I. Values during 24-hour control period

Variable	Maternal blood	Fetal blood	Fetal urine	Amniotic fluid
Arterial pH	7.44 ± 0.01	7.33 ± 0.01		
Pco ₂ (mm Hg)	38.4 ± 1.6	53.3 ± 1.1		E-1-400
Po ₂ (mm Hg)	108.1 ± 2.0	20.9 ± 1.1	***************************************	
Osmolality (mOsm/kg)	304.1 ± 1.8	302.3 ± 2.1	142.1 ± 8.0	274.9 ± 3.8
Na ⁺ (mmol/L)	145.0 ± 0.8	139.3 ± 0.8	41.7 ± 4.1	115.2 ± 4.1
Cl ⁻ (mmol/L)	114.6 ± 0.5	108.8 ± 0.7	35.8 ± 4.3	99.1 ± 4.4
K ⁺ (mmol/L)	4.4 ± 0.1	4.5 ± 0.1	5.7 ± 1.4	8.6 ± 1.3
Bicarbonate (mmol/L)	25.4 ± 0.6	27.0 ± 0.7	·	
Hematocrit (%)	28.7 ± 1.3	33.4 ± 1.9	-	
Plasma protein (gm/dl)	6.4 ± 0.2	3.6 ± 0.1		
Lactate (mmol/L)	0.6 ± 0.1	0.9 ± 0.1	0.10 ± 0.01	1.9 ± 0.4
Glucose (mmol/L)	2.8 ± 0.2	1.0 ± 0.1	0.10 ± 0.01	0.3 ± 0.1

Values are mean \pm SE, n = 7.

that seen in hydropic human fetuses with erythroblastosis. ^{1, 2} In one fetus the rate was lowered to 8.6 mmol/hr and in another the rate was increased to 11.3 mmol/hr to achieve the desired lactate concentration.

At each sample time, fetal arterial blood (3 ml), maternal arterial blood (1 ml), amniotic fluid (1 ml), and fetal urine (1 ml) were collected. Fetal blood removed during sampling was replaced with an equal volume of heparinized maternal blood. Urine flow rate (by direct collection after emptying the bladder⁷) and estimated amniotic plus allantoic fluid volume were determined at each sample time. The estimation of amniotic plus allantoic fluid volume by ultrasonography was accomplished by dividing the abdomen into four equal quadrants and scanning each quadrant for fluid pockets. The largest vertical column of fluid in each of the four quadrants was summed for an amniotic fluid index and was determined in triplicate.8 In sheep, this technique allows for estimation of the total amniotic plus allantoic fluid volumes and examination of the fetus for edema. Preliminary studies in humans and sheep have shown high correlations between the amniotic fluid index and directly measured amniotic fluid volumes.9 In this study each millimeter increase in the amniotic fluid volume corresponded to an increase in amniotic fluid volume of approximately 45 ml.

At the end of the experiment the animal was killed with pentobarbital sodium (100 mg/kg) and autopsy was performed. The amniotic plus allantoic fluid volume was measured at autopsy by direct collection, and fetal weight was determined.

Analytic measurements. All samples were analyzed for osmolality (Advanced Instruments, model 3D2), Na⁺, Cl⁻, and K⁺ (Nova Biomedical, model 5+5 electrolyte analyzer), lactate, and glucose (YSI model 2300). Maternal and fetal blood samples were also analyzed for pH, PO₂, and PCO₂ (39° and 39.5° C, respectively (Instrumentation Laboratories, model 1302), hematocrit in triplicate, and plasma protein (Reichert TS meter).

Data analysis and calculations. All data are pre-

sented as mean \pm SE or as a mean change \pm SE from the average value during the control period, unless otherwise specified. Data were analyzed by two-way analysis of variance for repeated measures with p < 0.05 considered statistically significant. Values in the results are averages during the 3 days of sodium lactate infusion unless specified otherwise.

The change in fetal blood volume was estimated from the hematocrit, as previously described. ¹⁰ This technique is valid because the ovine fetus does not have a releasable store of red blood cells. ¹⁰ The technique also has the advantage that changes in red blood cell mass with growth are taken into account, and only deviations from the normal increase are detected. Estimated fetal extracellular fluid volume was calculated from plasma protein concentrations. ¹¹ This calculation is based on the assumption that plasma proteins rapidly distribute between the interstitial space and plasma because of a capillary permeability to proteins in the fetus fifteen times that of the adult. ¹² The accuracy of this estimation is unknown. However, large changes in extracellular fluid volume should be readily detected.

Maternal and fetal bicarbonate were calculated from the respective PCO_2 and pH with the equation¹³: Bicarbonate = $0.029131 \times PCO_2^{(-5.0844 + 0.6854 \text{ pH} + 0.0242 \text{ pH}^2)}$ (corrected to 39.5° C).

Results

The mean values for all variables during the 24-hour control period are presented in Table I. These values are within the normal range for late-gestation fetuses as measured in our laboratory. Control urine flow was 0.64 ± 0.10 ml/min (mean \pm SE), and fetal weight at autopsy was 3.5 ± 0.3 kg at a gestational age of 135 ± 1 days.

The infusion of 10 mmol/hr sodium lactate (pH 5.0) into the fetal circulation resulted in a slight alkalosis (Fig. 2, A), with the mean increase in fetal pH of 0.10 ± 0.01 pH units (p < 0.0001) during the 3-day infusion. As seen in Fig. 2, B and C, the fetal PCo₂

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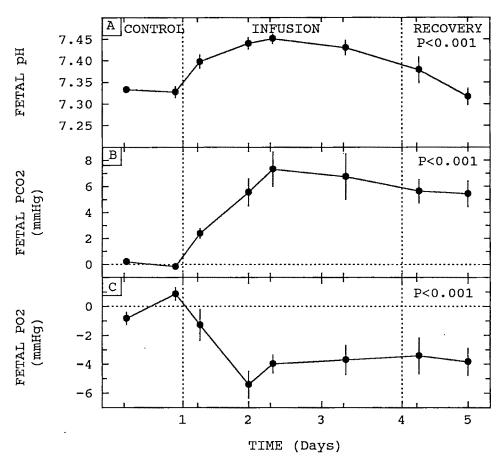


Fig. 2. Mean \pm SE fetal pH (A) and mean \pm SE changes from control in PCO₂ (B) and PO₂ (C) during infusion of 5 mol/L sodium lactate to fetus.

increased (5.5 \pm 1.2 mm Hg, p < 0.0001) and the Po₂ decreased (3.6 \pm 0.9 mm Hg, p < 0.0001) during the infusion.

Fig. 3 is a summary of the changes from control levels in fetal plasma composition. Fetal plasma osmolality (Fig. 3, A) rose by a mean of 7.8 \pm 1.4 mOsm/kg (p < 0.0001) over the 3-day infusion. Fetal Na⁺ (Fig. 3, B) increased only slightly (1.4 \pm 0.5 mEq/L, p < 0.001) and fetal Cl⁻ (Fig. 3, C) decreased dramatically (14.6 \pm 2.2 mEq/L, p < 0.0001) during the sodium lactate infusion. Lactate (Fig. 3, D) increased by a mean of 6.0 \pm 0.9 mmol/L (p < 0.0001). Fetal plasma bicarbonate (Fig. 3, E) also was elevated (10.9 \pm 1.2 mmol/L, p < 0.0001). In addition to these changes, fetal plasma K⁺ decreased by 1.04 \pm 0.09 mEq/L (p < 0.0001), and glucose did not change.

None of the maternal variables showed a statistically significant change during the 72-hour sodium lactate infusion. However, the maternal lactate decreased by 0.20 \pm 0.09 mmol/L during the infusion (p=0.06). In addition, maternal plasma Cl⁻ was significantly reduced during the recovery period (3.4 \pm 1.7 mEq/L, p<0.006).

The osmotic and concentration gradients across the

placenta (fetal minus maternal) are shown in Fig. 4. A reversal of the transplacental osmotic gradient (from -1.8 ± 1.3 to $+7.5 \pm 1.9$ mOsm/kg) in favor of increased fluid acquisition by the fetus is seen in Fig. 4, A. The infusion of sodium lactate resulted in a reduction (27%) in the transplacental Na+ concentration gradient but not a reversal (Fig. 4, B). The Cl- concentration gradient across the placenta was the most significantly enhanced of the electrolyte gradients (Fig. 4, C). Control fetal Cl⁻ was -5.8 ± 0.9 mEq/L lower than maternal Cl-. As the fetal Cl- decreased during the infusion, the Cl- gradient across the placenta increased to an average of -19.5 ± 2.6 mEq/L over the 3-day infusion. A small concentration gradient of 0.35 ± 0.18 mmol/L existed for lactate in the control period and, as the lactate increased during the 3-day infusion, the concentration gradient toward the mother was increased to $6.6 \pm 0.9 \, \text{mmol/L}$ (Fig. 4, D). Finally, the transplacental bicarbonate concentration gradient was significantly increased (1.6 \pm 0.9 to 12.5 \pm 1.1 mEq/L, p < 0.0001) (Fig. 4, E) favoring loss of bicarbonate to the maternal circulation.

Fetal blood volume as seen in Fig. 5, A, showed no change during the first 2 days of infusion. During the

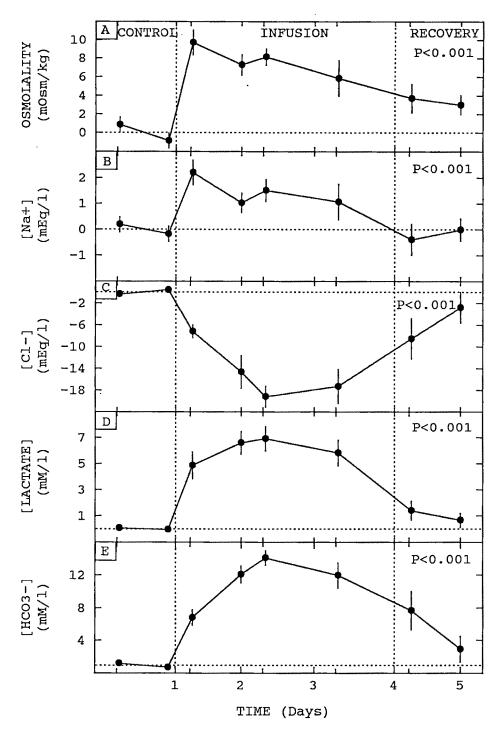


Fig. 3. Mean ± SE changes from control in fetal blood osmolality (A), Na* (B), Cl- (C), lactate (D), and bicarbonate (E) during sodium lactate infusion.

third day and throughout the recovery period, blood volume decreased significantly (p < 0.0001), averaging $9.9\% \pm 2.5\%$ below control at the end of the recovery period. Changes in estimated fetal extracellular fluid volume underwent a pattern similar to the changes seen in blood volume (Fig. 5, B) except that the rise in extracellular fluid volume during the infusion period $(7.8\% \pm 2.3\%)$ was statistically significant (p < 0.0001),

and the decline during the recovery period was not as extensive (5.8% \pm 2.3%). No ascites or pleural effusions were seen during the daily ultrasonography examinations.

Urine composition changes are shown in Fig. 6. Although the urine osmolality (Fig. 6, A) rose by a mean of $66.4 \pm 9.5 \text{ mOsm/kg}$ (p < 0.0001) during the infusion, the urine remained hypotonic relative to fetal 1600 Powell and Brace

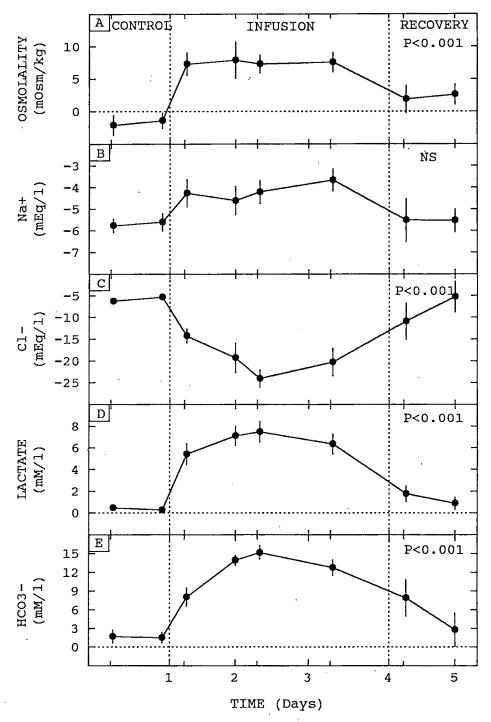


Fig. 4. Mean \pm SE in transplacental (fetal minus maternal) osmotic (A) and concentration gradients for Na⁺ (B), Cl⁻ (C), lactate (D), and bicarbonate (E) during 5 mol/L sodium lactate infusion.

blood throughout the experiment. Urine Na⁺ (Fig. 6, B) accounted for the majority of the increase in osmolality as it increased by 46.4 ± 4.5 mEq/L (p < 0.0001), whereas urine lactate (Fig. 6, D) increased by only 9.7 ± 3.1 mmol/L (p < 0.0001). Urine Cl⁻ (Fig. 6, C) was not significantly altered during the infusion. The mean control value for urine pH was 7.32 ± 0.15 , and the urine pH rose by 0.73 ± 0.17 pH

units during the infusion (p < 0.0001). In addition to the changes seen in urine composition, the urine production rate (Fig. 7, A) increased by a mean of 1.9 ± 0.4 L/day (p < 0.0001). As seen in Fig. 7, B, all the infused Na⁺ was excreted in the urine (10.8 ± 1.9 mEq/hr mean increase above control). Lactate excretion (Fig. 7, D), on the other hand, rose by 1.1 ± 0.3 mmol/hr, indicating that only 11.4% of the infused

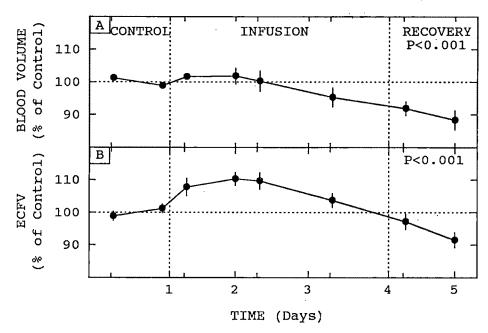


Fig. 5. Mean ± SE fetal blood volume (A) and estimated extracellular fluid volume (ECFV) (B) expressed as percent of control.

lactate was excreted in the urine. Urinary Cl- excretion (Fig. 7, C) rose by 5.0 ± 0.9 mEq/hr during the infusion.

Amniotic fluid osmolality (Fig. 8, A) remained unchanged throughout the procedure. However, amniotic Na⁺ $(6.4 \pm 2.1 \text{ mEq/L}, p < 0.001)$ and lactate $(7.9 \pm 2.0 \text{ mEq/L}, p < 0.001)$ increased (Figs. 8, B and D) and remained elevated throughout the recovery period. Amniotic fluid Cl- decreased by a mean of $15.6 \pm 3.7 \text{ mEq/L}$ (p < 0.0001). In addition, the amniotic fluid index (Fig. 9) rose throughout the duration of the experiment and remained elevated during the recovery period (85% \pm 14%, p < 0.0001). At autopsy a mean of 5.3 ± 0.8 L of amniotic plus allantoic fluid was collected from the uterine compartment. This volume would contain roughly 650 mmol Na+, 70 mmol lactate and, 450 mmol Cl,- on the basis of amniotic fluid concentrations at the final sample. Compared with the approximately 720 mmol that was infused, this accounts for roughly 77.6% and 9.6% of the infused Na+ and lactate, respectively.

Comment

The underlying mechanisms that produce hydrops fetalis or polyhydramnios in the human fetus are unknown. The data from two recent reports1.2 suggest that hydrops occurs during erythroblastosis fetalis only in those fetuses with elevated plasma lactate concentrations. This observation led us to hypothesize that the elevated lactate level could produce these fetal fluid imbalances because it could act osmotically to draw water from the maternal to the fetal compartment. This is supported by Hansen and Gest¹⁴ in a review of hydrops fetal in which they suggest that osmotically active substances accumulate in the blood of the fetus when oxygen delivery is reduced. The results of our study support this hypothesis, in that the infusion of 5 mmol/L sodium lactate for 3 days resulted in extensive polyhydramnios. Urine production increased by 1.9 ± 0.4 L/day above control. Over 3 days of infusion this would be an increase of 5.7 ± 1.2 L. The amniotic plus allantoic fluid volume measured at autopsy was 5.3 ± 0.8 L, compared with a normal of 0.5 to 1.0 L.¹⁵ Therefore large amounts of fluid were crossing the placenta, entering the fetal vasculature, and being excreted in the form of dilute urine, which remained in the amniotic and allantoic fluid compartments.

The driving force for this fluid accumulation could be either the general effects of an increase in fetal osmolality or the specific effects of an elevated fetal plasma lactate level. In a recent study in which we infused comparable amounts of sodium chloride into fetal sheep for 3 days, neither polyhydramnios nor hydrops developed.6 Increases in fetal osmolality and Na+ with sodium chloride infusion were similar to those observed in this study. Additionally, urine production increased to 3.5 L/day during the sodium chloride infusion. Thus it appears that the presently observed accumulation of 4 to 5 liters of excess fluid was due to the specific effects of the lactate. This is consistent with previous reports suggesting that Na+ and Cl- cross the placenta¹⁵ but that lactate does not.¹⁷ Other osmotic agents have not, to our knowledge, been examined for their ability to produce polyhydramnios or hydrops fe1602 Powell and Brace December 1991
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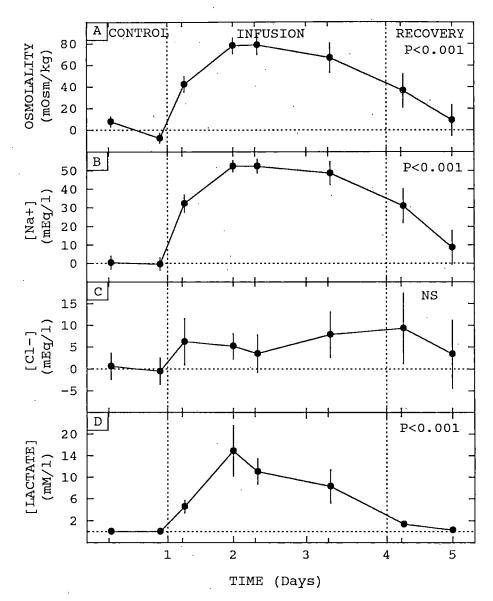


Fig. 6. Mean \pm SE changes in fetal urine osmolality (A), Na⁺ (B), Cl⁻ (C), and lactate (D) during infusion of sodium lactate to fetus.

talis during long-term infusions. It seems likely that other osmotically active substances that have limited placental permeability and are effectively excreted by the fetal kidney would lead to polyhydramnios, e.g., mannitol. However, infusion of sodium chloride did not result in polyhydramnios,⁶ most likely because sodium chloride more readily crosses the placenta.

It should be questioned whether the observed fluid accumulation is consistent with that predicted from the placental filtration coefficient and changes in placental lactate. The filtration coefficient for the sheep placenta has been estimated to be 0.0067¹⁸ or 0.026¹⁹ ml/min/kg/mm Hg. On the basis of an increase in lactate of 6.6 mmol/L, the total amount of water crossing the placenta would be 13.2 or 51.2 L over 3 days. Clearly the observed volume accumulation is not out-

side of the range predicted and further suggests that factors other than lactate are involved in water transport across the placenta during this experiment.

The changes in fetal Cl⁻ during the sodium lactate infusion were quite dramatic. The decreased Cl⁻ resulted in the largest steady-state transplacental Cl⁻ concentration gradient ever reported in the sheep. The increased fetal urine flow was associated with a 5.0 mEq/hr increase in Cl⁻ excretion even though urine Cl⁻ did not change. On the basis of plasma Cl⁻ changes, the loss of Cl⁻ from the fetal extracellular fluid compartment would be negligible compared with the total amount excreted. On the basis of mean Cl⁻ transplacental concentration gradient during the infusion, the published placental permeability coefficient for Cl⁻ (0.0098 ml/sec/kg),¹⁶ and the mean fetal weight at au-

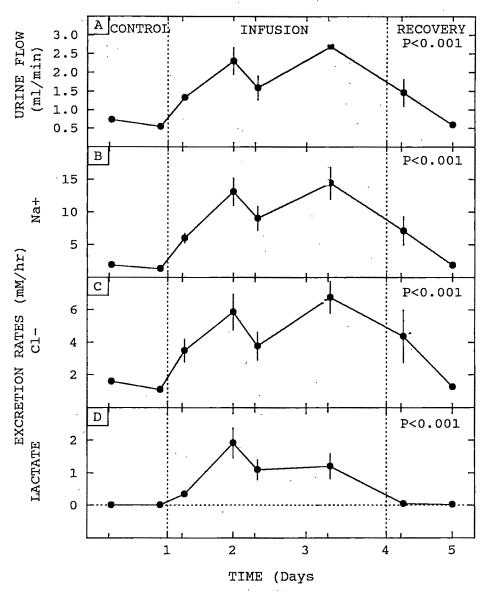


Fig. 7. Urine flow rate (A) and urinary excretion rate for Na⁺ (3), Cl⁻ (C), and lactate (D) during infusion of sodium lactate at a rate of 10 mmol/hr.

topsy, the transfer of CI- across the placenta would have been 2.4 mEq/hr. These data indicate that the drop in fetal plasma Cl- may have been due to a low placental permeability. However, to account for the observed renal excretion of Cl-, the permeability, of the placenta for Cl⁻ must be at least twice the previously reported value. This is also supported by the accumulation of 450 mEq of Cl⁻ in the amniotic plus allantoic compartments. Overall, it would appear that the fetus excretes the infused Na+ accompanied by several anions, i.e., lactate, bicarbonate, and Cl-.

In previous studies in which lactate tagged with carbon 14 was infused into fetal sheep, ≥90% of the infused lactate was metabolized20 when lactate levels were elevated. The increased Pco2, and bicarbonate and the decreased Po2 seen in this study suggest that similar amounts of lactate were metabolized. This is consistent with our observation that 10% of the infused lactate wa excreted in the fetal urine. Bicarbonate concentrations were greatly increased during the sodium lactate infision, and the fetuses became alkalotic. This is most lilely related to the metabolism of the infused lactate, b-cause metabolism of an anion produces an anion as a by-product. The decreased Cl- also may cause bicarbonate to increase. Urinary bicarbonate excretion was elevated on the basis increased of pH of the fetal urine. The increase in PCO2 would promote removal across the placenta of the metabolic by-products of l_ctate utilization. Furthermore, the elevated bicarbon te may be exerting an osmotic force for water accumulation across the placenta. This possibility is supported by a model for water transfer across the

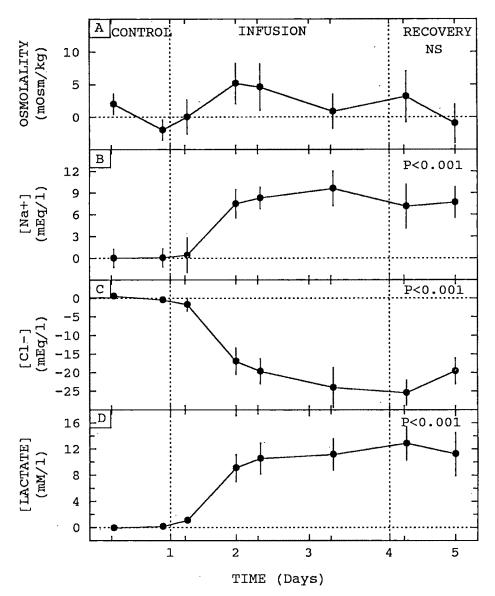


Fig. 8. Amniotic fluid osmolality (A), Na⁺ (B), Cl⁻ (C), and lactate (D) expressed as mean \pm SE change from control during infusion of sodium lactate.

human placenta developed by Wilbur et al.,21 in which bicarbonate was proposed as a major osmotic agent for water accumulation by the fetus. In their computer testing of this theory, bicarbonate and other substances that are present in higher concentrations in the fetus than in the mother (i.e., carbon dioxide, urea, fructose, and actively transported solutes such as Ca++ and amino acids) exerted the major driving force for water gain by the fetus. This theory was tested in a computer model for water transfer across the sheep placenta.22 In that evaluation Faber and Anderson²² concluded that the effects of changes in bicarbonate were for the most part counterbalanced by changes in the sodium chloride gradient across the placenta. Therefore, bicarbonate was thought to have only a modest effect on water transport.

The hypoxia experienced by these fetuses may have

been a sufficient stimulus to accelerate red blood cell production, because previous studies have suggested fetal red blood cell production can be augmented in as little as 3 to 5 days.23 We believe the decrease in the calculated blood volume on the third day of infusion and throughout the recovery period may be to an increased red blood cell volume relative to plasma volume and does not necessarily reflect a true decrease in blood volume. The 8% increase in estimated extracellular fluid volume observed in this study relative to the lack of change initially in blood volume suggests a true increase in extracellular fluid volume but does not represent extensive edema formation. This is supported by the fact that fetuses were examined daily by ultrasonography and no ascites or pleural effusions were observed.

In human fetuses with isoimmunization, hydrops will

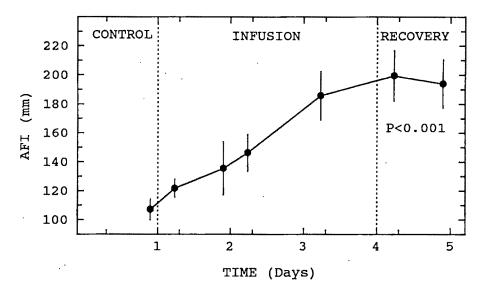


Fig. 9. Amniotic fluid index (AFI) responses of amniotic plus allantoic fluid volume by ultrasonography during infusion of sodium lactate.

develop in approximately 25%, and 75% of those will have polyhydramnios.¹⁴ In addition, polyhydramnios often occurs with mild to moderate edema associated with anemia²⁴ and sometimes precedes the development of hydrops. In our study we expected hydrops to develop in the sheep fetuses but found mild edema at most and massive polyhydramnios. It is possible that, given more time, the fetuses in this study may have become edematous. On the other hand, the tremendous ability of urine production to increase in the nearterm sheep fetus would tend to promote the development of polyhydramnios, rather than hydrops, in this study. The fact that edema develops in human fetuses suggests that the kidney of the erythroblastotic human fetus with an elevated lactate level may not be able to excrete excess fluid as effectively as that of the nearterm sheep fetus. In addition, the stress of hypoxia caused by anemia may result in elevated arginine vasopressin concentrations in the human fetus, thereby producing an antidiuresis that would contribute to hydrops formation. When the sheep fetus experiences severe hypoxemia, there is an increase in arterial pressure, a decrease in renal blood flow, and an increase in renal vascular resistance.25 These changes are associated with increases in plasma epinephrine, arginine vasopressin, and plasma renin activity25 and could promote edema formation. Because the fetuses in this study had only mild hypoxia and their renal function was not impaired, as evidenced by the increased urine production, polyhydramnios rather than hydrops developed.

This study represents an attempt to isolate the osmotic effects of increased lactate seen in hydropic human fetuses. Although there is no evidence that the infusion of sodium lactate will mimic the conditions of severely anemic fetuses, we believe that lactate is a

powerful osmotic agent in the fetus, although other factors are clearly involved in altering the fluid distribution. For example, there are circumstances in both sheep and human fetuses in which the plasma lactate level is elevated with no indication of either hydrops or polyhydramnios.26-30 Hypoxemia in sheep fetuses results in an increase in fetal lactate concentration, but changes in amniotic fluid volume were not explored in these studies. The lack of hydrops formation with elevated lactate levels in hypoxic fetuses and the results of our study suggest that elevated plasma lactate levels alone may not account for hydrops formation in erythroblastotic human fetuses. Other components necessary for the development of hydrops in erythroblastosis remain unknown. However, we have shown that lactate can act osmotically to increase the fluid content of the fetal compartment. This, along with other physiologic alterations occurring in severely anemic fetuses, such as reduced renal function and elevated vasoactive or antidiuretic hormone concentrations, could explain the formation of hydrops in these fetuses.

The amniotic fluid osmolality did not change even though extensive fluid accumulation occurred. This is rather surprising in light of the tremendous volume of dilute urine that entered that compartment during the infusion. The amniotic fluid compositional changes indicate nearly all of the excreted lactate remained in the amniotic fluid. It would appear that bicarbonate also was elevated and that these two anions compensated for the decreased Cl... The fact that amniotic plus allantoic fluid volume was elevated in response to sodium lactate but not sodium chloride infusion⁶ suggests that the lactate and bicarbonate may be acting osmotically to retain fluid in the amniotic space. This may be due to a low permeability of the pathway for direct exchange of lactate between amniotic fluid and the fetal blood perfusing the intramembranous pathway.³¹ Our laboratory has shown that water directly enters the fetal vasculature from the amniotic fluid independent of fetal swallowing and that Na⁺ and Cl⁻ may move relatively easily across this pathway.¹⁹ By comparison, this study suggests that the intramembranous pathway may be relatively impermeable to lactate. This is supported by the fact that amniotic fluid lactate concentrations under basal conditions are higher than concentrations in fetal blood, fetal urine, or allantoic fluid and by the finding in this study that nearly all of the excreted lactate remained in the amniotic compartment.

The regulation of composition and volume of amniotic fluid is not clearly understood. It appears from the sodium chloride and sodium lactate infusion studies that amniotic fluid osmolality is conserved in the face of tremendous changes in volume or fetal urine flow rate. The entry of large volumes of urine and perhaps some lung secretions and direct exchange between the fetal blood and amniotic fluid across the fetal membranes all contribute to varying degrees. From this study, it appears that amniotic Cl⁻ was not tightly regulated as Cl⁻ decreased, even though the urinary excretion of Cl⁻ was increased. Alternatively, as lactate and bicarbonate accumulated in the amniotic fluid, the Cl⁻ may have decreased to maintain electrical neutrality.

In conclusion, this study suggests that alterations in fetal plasma lactate concentration may be a major determinant of fetal fluid imbalances. Moderate, sustained elevations in fetal plasma lactate concentration proved to be a powerful osmotic agent in the sheep fetus, in that large volumes of fluid were accumulated in the fetal compartment. This study demonstrates the accumulation of fluid in the fetal compartment during sodium lactate infusion was due specifically to lactate or its metabolic by-products because comparable infusions of sodium chloride did not result in fluid accumulation. Thus elevations in fetal lactate concentration and its metabolic by-products may be the primary underlying mechanism for fetal fluid imbalances seen in erythroblastotic human fetuses.

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Left thoracic duct lymph flow responses to angiotensin II or atrial natriuretic factor infusion in the ovine fetus

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In the ovine fetus it is known that left thoracic duct lymph flow rate relative to body weight is four to five times adult levels, but it is not known whether the circulating hormones modulate fetal lymph flow. To explore this, we intravenously infused either angiotensin II (10 to 400 ng/min, n = 8) or atrial natriuretic factor (500 to 1000 ng/min, n = 8) into chronically catheterized fetal sheep for 30 minutes. Significant increases occurred in fetal arterial (p < 0.0001) and venous (p = 0.018) pressures during the angiotensin II infusion, and thoracic duct lymph flow rate underwent a dose-dependent increase (r = 0.888, p = 0.0033). With termination of the angiotensin II infusion, fetal vascular pressures rapidly returned to control levels, and lymph flow fell from 18.8% ± 10.1% (mean ± SE) above control to 13.7% ± 7.7% below preinfusion levels (ρ < 0.01). During the atrial natriuretic factor infusion, fetal arterial pressure and circulating blood volume decreased significantly (p < 0.01), whereas thoracic duct lymph flow was unchanged. After termination of the atrial natriuretic factor infusion, fetal arterial pressure returned toward control, blood volume remained reduced, and lymph flow rate underwent a transient rise to $35.6\% \pm 15.7\%$ (p < 0.05) above control levels. These data suggest that angiotensin II and atrial natriuretic factor have significant but opposite effects on fetal thoracic duct lymph flow rate, with angiotensin II stimulating and atrial natriuretic factor suppressing lymph flow. (AM J OBSTET GYNECOL 1991;165:1607-13.)

Key words: Fetus, lymphatics, arterial pressure, venous pressure, blood volume

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The lymphatic system plays an important role in maintaining fluid balance by returning both fluid and protein to the circulation. Although the lymphatic system of the adult has been explored in a large number of studies,1 relatively little attention has been paid to the lymphatic system of the fetus. It is known that in the chronically catheterized ovine fetus left thoracic duct lymph flow rate averages four to five times adult levels relative to body weight.2,3 It also is known that 508 Brace and Andres December 1991
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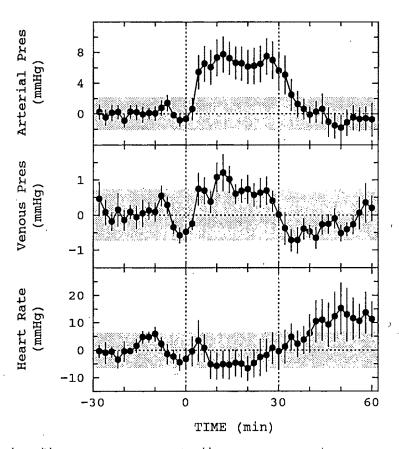


Fig. 1. Fetal arterial pressure, venous pressure, and heart rate responses to intravenous angiotensin II infusion ($210 \pm 60 \text{ ng/min}$, n = 8) from 0 to 30 minutes. Data are mean \pm SE change from mean value during 30-minute preinfusion period. Shaded area, 95% Confidence interval around preinfusion value.

Table I. Control fetal values during 30-minute preinfusion period

Variable	Mean ± SE*
Arterial pressure (mm Hg)	47.9 ± 1.4
Venous pressure (mm Hg)	3.0 ± 0.2
Heart rate (beats/min)	160 ± 5
Lymph flow rate (ml/min)	0.58 ± 0.07
Arterial pH	7.340 ± 0.015
Carbon dioxide tension (mm Hg)	54.2 ± 1.8
Oxygen tension (mm Hg)	23.7 ± 1.0
Hematocrit (%)	35.9 ± 1.3
Plasma protein concentration (gm/dl)	3.63 ± 0.08
Lymph protein concentration (gm/dl)	2.56 ± 0.09

*Combined data from angiotensin II— and ANF-infused fetuses; n = 16 for continuously measured variables and n = 12 for data derived from blood and lymph samples.

fetal thoracic duct lymph flow rate increases after vascular volume loading³ and decreases with elevations in venous pressure.^{4,5} In the adult, a variety of vasoactive hormones, including angiotensin II and the catecholamines,^{1,6,7} have been reported to alter lymph flow or lymphatic function. In addition, atrial natriuretic factor (ANF) has been reported to decrease the contractility of lymphatic vessels isolated from lambs.⁸ These observations, coupled with the fact that the above hormones have cardiovascular actions in the fetus similar to those in the adult, raise the possibility that vasoactive hormones may modulate lymphatic function in the fetus. To explore this hypothesis, we infused angiotensin II or ANF into chronically catheterized ovine fetuses while simultaneously measuring left thoracic duct lymph flow rate.

Material and methods

Animal preparation and maintenance. Sixteen fetal sheep at 126 to 133 days' gestation at the time of surgery were surgically prepared and maintained postoperatively as previously described in detail elsewhere.² Briefly, with the animals under gas inhalation anesthesia and with strict aseptic techniques, catheters were placed in the fetal descending and ascending aorta, inferior and superior vena cava, and amniotic space. An additional large-diameter catheter was placed in the left thoracic lymph duct at the base of the neck just distal to its junction with the left cervical and brachiocephalic lymph ducts and jugular vein.² Postoperatively, the lymphatic catheter was attached to the su-

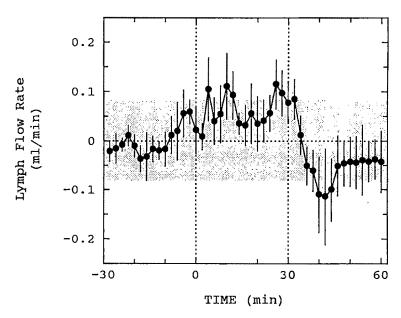


Fig. 2. Fetal left thoracic duct lymph flow rate response to intravenous angiotensin II infusion $(210 \pm 60 \text{ ng/min}, n = 8)$ from 0 to 30 minutes. Data are mean \pm SE change from mean value during 30-minute preinfusion period. Shaded area, 95% Confidence interval around preinfusion

perior vena caval catheter so that lymph spontaneously returned to the circulation. The animals were maintained on a regimen of prophylactic antibiotics for 5 days as previously described,² and experiments were conducted on postsurgical days 5 through 11. This study was approved by the Animal Subjects Committee of the University of California, San Diego, and we followed the guidelines for care and use of laboratory animals as approved by our institution.

Experimental protocol and measurements. The protocol consisted of a 30-minute control period and a 30minute infusion period, followed by a 30-minute recovery period. During the infusion period, either angiotensin II (10 [n = 1], 50 [n = 1], 100 [n = 2], 200 [n = 1], or 400 [n = 3] ng/min) or ANF (500 [n = 2]or 1000 [n = 6] ng/min) was infused into the fetal inferior vena cava in a volume of 0.2 to 0.4 ml/min of isotonic saline solution. At the start of the infusion, 0.8 ml of the infusate was injected into the infusion catheter (volume, 1.2 ml) to reduce the catheter dead space.

Throughout the experiment, fetal arterial and venous pressures, amniotic fluid pressure, heart rate, and left thoracic duct lymph flow rate were measured continuously as previously described.2,9 Fetal vascular pressures were referenced to amniotic fluid pressure,9 and lymph flow was calculated 30 times per second and displayed on a polygraph recorder with an on-line computer.² Data were stored on disk at 30-second intervals with the on-line computer for later data reduction and analyses.

In 12 of the 16 fetuses at 10 and 25 minutes during

the control, infusion, and recovery periods, 1 ml samples of fetal arterial blood were collected for measurement of hematocrit^{10, 11} plasma protein concentration (TS meter, American Optical Corp., Buffalo, N.Y.), and blood gases and pH (model 1302 blood gas analyzer, Instrumentation Laboratory, Inc., Lexington, Mass.). A sample of lymph was collected at the same time for measurement of lymph protein concentration. Changes in fetal blood volume were calculated from hematocrits as previously described. 10,11 This technique is valid because the late-gestation ovine fetus does not have a pool of releasable red blood cells10 such as occurs in the spleen of the adult.

Data analysis. The data are presented as the mean ± SE. Mean values for each variable during the control period were calculated for both groups of animals and compared with an unpaired t test. Because there were no differences between the angiotensin II-and ANF-infused fetuses during the control period, the data were combined. In the figures the data are plotted either as the change from or as a percent of mean control values. Both parametric and nonparametric two-factor analyses of variance for repeated measures were used to determine the statistical significance of the changes with time in each variable. A value of $p \le 0.05$ was assumed to represent statistical significance, but we also present p values from 0.05 to 0.10 because of the potential for dependency of response on infusion rate. If there were statistically significant changes with time ($p \le 0.05$), the 95% confidence interval about the mean control value was plotted as

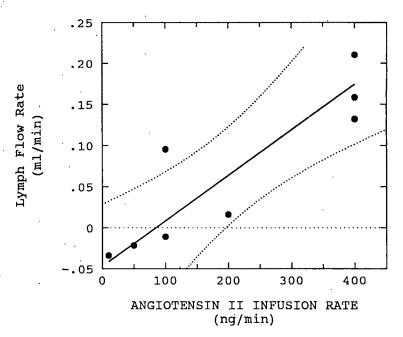


Fig. 3. Thoracic duct lymph flow response in individual fetuses as function of angiotensin II infusion rate (r = 0.888, p = 0.0033). Each *dot* represents mean during last 20 minutes of 30-minute angiotensin II infusion. Regression line and its 95% confidence interval are shown.

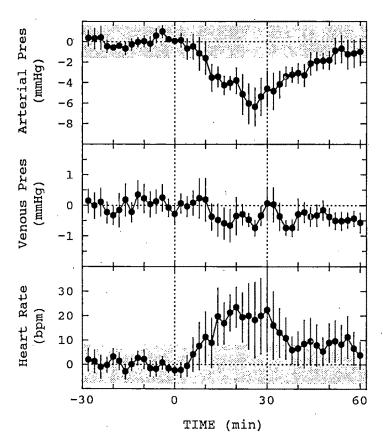


Fig. 4. Fetal arterial pressure, venous pressure, and heart rate responses to intravenous ANF infusion (880 \pm 80 ng/min, n=8) from 0 to 30 minutes. Data are mean \pm SE change from mean value during 30-minute preinfusion period. Shaded area, 95% Confidence interval around preinfusion value.

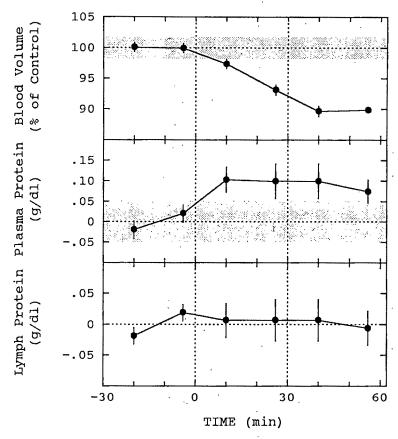


Fig. 5. Fetal blood volume, plasma protein concentration, and lymph protein concentration changes in response to intravenous ANF infusion (880 \pm 80 ng/min, n=8) from 0 to 30 minutes. Data are mean \pm SE relative to value during 30-minute preinfusion period. Shaded area, 95% Confidence interval about mean value during preinfusion period.

 $\pm t_{0.05} \times \sqrt{2 \text{MSE}/n}$, where MSE is the mean square error as determined from the analysis of variance and n is the number of experiments. Bivariate and multivariate regression analysis was used to explore the statistical relationship among variables.

Results

The fetuses averaged 138 days' gestation at the time of experimentation. Mean values during the 30-minute preinfusion period are given in Table I. These values are consistent with previous reports from this laboratory.^{2-1, 10}

Angiotensin II infusion. Angiotensin II was infused at a mean rate of 210 ± 60 ng/min for 30 minutes. As seen in Fig. 1, this produced an elevation in fetal arterial (p < 0.0001) and venous (p = 0.018) pressures during the infusion, with pressures rapidly returning to control levels after the infusion was terminated. The fetal heart rate response to angiotensin II infusion was significant (p < 0.01), two-factor analysis of variance), but this was due to an increase during the recovery period. Heart rate tended to decrease during the low angio-

tensin II infusion rates and increase during the high infusion rates, with no net change occurring during the infusion (Fig. 1). These responses are consistent with the dose-dependent responses to angiotensin II recently reported.¹²

Fetal thoracic duct lymph flow rate tended to increase during the angiotensin II infusion, averaging 20% above control during the last 5 minutes of the infusion (Fig. 2). This increase was dependent on the infusion rate (Fig. 3) in that the lymph flow rate consistently increased only with >100 ng/min of angiotensin II. From multivariate regression analysis, the lymph flow response was dependent on angiotensin II infusion rate (p = 0.0057) and heart rate change (p = 0.032) simultaneously (p = 0.960) but not on the arterial or venous pressure changes. When the angiotensin II infusion was terminated, lymph flow rate decreased rapidly from $18.8\% \pm 10.1\%$ above control to $13.7\% \pm 7.7\%$ below preinfusion levels (p < 0.01).

Fetal arterial pH, carbon dioxide and oxygen tensions, blood volume, and plasma protein concentrations did not change significantly during or after the angio-

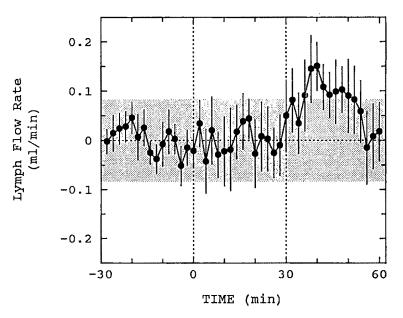


Fig. 6. Fetal left thoracic duct lymph flow rate response to intravenous ANF infusion (880 \pm 80 ng/min, n = 8) from 0 to 30 minutes. Data are mean \pm SE change from mean value during 30-minute preinfusion period. Shaded area, 95% Confidence interval around preinfusion value.

tensin II infusions. Lymph protein concentration underwent a nonsignificant decrease of 0.17 ± 0.07 gm/dl (p = 0.051) by the end of the angiotensin II infusion.

ANF infusion. The fetal cardiovascular responses to 500 to 1000 ng/min of ANF infusion are shown in Fig. 4. Fetal arterial pressure decreased by an average of 6 mm Hg during the infusion (p < 0.001), whereas venous pressure was unchanged and heart rate increased 20 beats/min (p < 0.01). Fetal blood volume decreased (p < 0.0001) in parallel with the fall in arterial pressure (Fig. 5), averaging 10% below normal throughout the recovery period. Plasma protein concentration underwent a small but significant increase (p = 0.0068) of 0.10 ± 0.04 gm/dl during the ANF infusion, whereas lymph protein concentration was unchanged (Fig. 5). Arterial oxygen and carbon dioxide tensions were unchanged during the ANF infusion, whereas pH decreased by 0.016 ± 0.004 units (p = 0.057 parametric, p < 0.05 nonparametric analysis of variance). Oxygen tension increased by 2 mm Hg during the recovery period (p = 0.0011), whereas pH remained reduced.

Even though ANF infusion significantly altered cardiovascular function, fetal left thoracic duct lymph flow rate was not altered during the ANF infusion (Fig. 6). However, with termination of the ANF infusion, lymph flow rapidly increased (p < 0.01) to a maximum of $35.6\% \pm 15.7\%$ above control (Fig. 6). Lymph flow was elevated above control for 20 minutes before returning to basal levels.

Comment

In our study we sought to determine whether the vasoactive hormones angiotensin II and ANF alter lymphatic function in the chronically catheterized ovine fetus. One of the difficulties in studying lymphatic function in the sheep fetus is that lymph flow rate is highly variable on a minute-to-minute basis.² In spite of this, our study provides evidence suggesting that both of these hormones may modulate lymphatic function in the ovine fetus.

During angiotensin II infusion, fetal lymph flow increased. In addition, when the angiotensin II infusion was terminated, fetal lymph flow decreased rapidly in parallel with the falls in arterial and venous pressure. Because these changes in lymph flow occurred independent of blood volume changes, the rise and fall in lymph flow would be consistent with a direct stimulatory effect of angiotensin II on the fetal lymphatic system. Because angiotensin II infusion has been shown to augment thoracic duct lymph flow rate in adult dogs and sheep, 6,7 it appears that the lymph flow response to angiotensin II in late-gestation fetal sheep may be similar to that of the adult.

During ANF infusion, fetal lymph flow rate was unchanged. However, there was a simultaneous decrease in blood volume of approximately 10%. This ANF-mediated decrease in blood volume is independent of renal excretion^{13, 14} and is due to a translocation of fluid from the plasma to the interstitial space; it occurs in both the adult^{13, 14} and fetus.^{15, 16} Because interstitial volume expansion (produced by vascular volume loading)

has been shown to increase fetal thoracic duct lymph flow rate,3 an increase in lymph flow would be expected from the increase in interstitial volume if ANF did not alter fetal lymphatic function. Thus the lack of an increase in lymph flow during the ANF infusion suggests that it suppressed lymphatic flow. This is consistent with a recent report in which ANF reduced the frequency and the amplitude of contractions in lymphatic vessels isolated from lambs.8 In addition, in our study, when the ANF infusion was terminated, there was an elevation in lymph flow rate. Because no volume shifts occurred at this time and because ANF is rapidly cleared from the fetal circulation,15-18 the rise in lymph flow occurred at the time when plasma ANF concentration would be returning to normal. This further supports the idea that ANF suppressed lymphatic function in the ovine fetus. There also may be regional or species differences in the effects of ANF on lymphatic function, in that lymph flow of the dog forelimb was unresponsive to intraarterial ANF infusion.19 However, there were no changes in interstitial volume in the latter study (as determined by weight changes), so that lack of a lymph flow response to ANF in the dog forelimb19 and the present study are not necessarily inconsistent.

In summary, the results of this study suggest that angiotensin II may stimulate and ANF may suppress lymphatic function in the ovine fetus.

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Oral-nasal membranes are not the major route for fetal absorption of amniotic fluid arginine vasopressin

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Intraamniotically injected substances such as arginine vasopressin and digoxin have been found to rapidly appear in the ovine fetal circulation, irrespective of whether the fetal esophagus has been ligated or occluded. To determine if the ovine fetal oral-nasal membrane plays a significant role in this fetal absorption of amniotic substances, we used two groups of chronically catheterized fetal sheep with a surgical glove sewed over the fetal head to prevent access of the head to amniotic fluid. In the first group 22.5 μg of arginine vasopressin was injected into the amniotic cavity; in the second group 22.5 μg was injected into the glove over the fetal head. We found that, after injection into the amniotic cavity, there were rapid and highly significant increases in amniotic fluid arginine vasopressin concentrations, from 6.1 \pm 1.3 to 51,249 \pm 18,182 pg/ml (mean \pm SE) (ρ < 0.00001). Concurrently there was a rapid increase in fetal plasma arginine vasopressin concentrations from 4.5 ± 1.3 to 93.8 ± 18.9 pg/ml (p < 0.00001). The increase was significant within 15 minutes and reached a maximum at 60 minutes after the injection. Fetal arterial pressure increased by 10 ± 2 mm Hg, whereas heart rate decreased by 30 \pm 5 beats/min (ρ < 0.00001). In contrast, after the injection into the glove covering the fetal head, there were no significant changes in any of the measured parameters. This suggests that the ovine fetal oral-nasal membrane is not a significant route of absorption of amniotic fluid arginine vasopressin and that the most likely route of absorption is the vascularized fetal surface of the placenta and vascularized fetal membranes, i.e., the intramembranous pathway. (AM J OBSTET GYNECOL 1991;165:1614-20.)

Key words: Vasopressin, amniotic fluid, intramembranous pathway, amnion, chorion

We recently described a pathway, the intramembranous pathway, for the movement of water and arginine vasopressin from the ovine amniotic cavity directly into the fetal circulation within the vascularized fetal membranes and fetal surface of the placenta.1,2 The intramembranous pathway also helped explain the finding of fetal urination being greater than fetal swallowing in many animal and human studies. In fact, we estimated that roughly 200 ml per day of amniotic water would enter the circulation of late-gestation ovine fetuses directly from the amniotic cavity under normal physiologic conditions.1 This intramembranous pathway appears to be the major route for the recirculation of amniotic fluid arginine vasopressin to the fetal circulation, because its absorption was not inhibited by fetal esophageal ligation.2

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Digoxin is a naturally occurring plant alkaloid that is used to treat cardiac arrhythmias in adults and fetuses. Hamamoto et al.3 injected digoxin into the amniotic cavity of fetal sheep with an esophageal occluder in place. They found that the digoxin was taken up rapidly by the fetal circulation, with 10 of 11 animals showing higher plasma levels in the descending aorta than in the umbilical vein. This observation conflicts with the expectation that umbilical vein concentrations would be greater than that in the descending aorta if the digoxin were absorbed by the intramembranous pathway, because the latter empties into the umbilical vein. The observation is consistent, however, with that expected if the digoxin were absorbed by the oral-nasal mucosa of the fetus.

In adults the oral and nasal mucosal membranes are highly vascularized and are used as a route of administration of certain medications, including arginine vasopressin and nitroglycerin. In this study we have investigated whether the ovine fetal oral-nasal membranes are a possible route for the absorption of amniotic fluid arginine vasopressin.

Methods

The protocol for this experiment was approved by the Animal Subjects Committee at our institution, and we followed the National Institutes of Health guidelines for the use and care of laboratory animals. Eight timedated pregnant sheep (Nebeker Ranch, Lancaster, Calif.) at a mean (\pm SEM) of 126 \pm 1 days' gestation at the time of surgery were used for this study. The surgical procedures, briefly described here, are detailed elsewhere.4 The animals were anesthetized with 1 gm thiamylal sodium injected intravenously and maintained with halothane (0.5% to 2%) in oxygen by an endotracheal tube. In the supine position, with a sterile surgical field, a midline incision was made in the abdominal wall, and the uterus was exposed through the abdominal incision. The fetal hind limbs were exteriorized through a uterine incision, and bilateral pedal arterial and saphenous venous catheters were placed and advanced to the level of the diaphragm. Amniotic fluid catheters were sutured to each hind leg. The fetal legs were returned to the uterus, and fetal amnion and chorion were ligated around the catheters twice to prevent any leakage of amniotic fluid. The uterus was closed in a water-tight fashion with a continuous suture.

The fetal head was then brought out through a second incision. A size 8 surgical glove was placed over the fetal head and sutured in a purse-string fashion to the base of the neck with a continuous suture loosely placed through the glove and skin of the neck. Size 8 was selected because it fit loosely over the head. One finger of the glove was cut and a catheter was placed into the area of the nose and ligated in place, thereby sealing the hole in the glove. The head was placed back into the uterus, and the fetal membranes and uterus were closed in a water-tight manner as described above. Maternal femoral arterial and venous catheters were placed in the right groin and advanced to the level of the maternal diaphragm. All maternal and fetal catheters were tunneled subcutaneously into a pouch sewn on the flank of the ewe.

The vascular catheters were flushed daily with heparin sodium (1000 U/ml) to maintain patency. Antibiotics (1 gm ampicillin [Omnipen, Wyeth Laboratories, Philadelphia]) were instilled daily into the amniotic cavity and intramuscularly (800,000 U of penicillin G and 1 gm dihydrostreptomycin) to the ewe during the postoperative preexperimental period.

Experiments were begun at least 5 days after surgery. The fetuses were monitored continuously for heart rate and arterial, venous, and amniotic fluid pressures. After a stable 30-minute control period, 18 ml of warmed saline solution containing 22.5 µg of arginine vasopressin was injected into the amniotic cavity of four fetuses. The injection catheter was flushed with 50 ml of amniotic fluid, and the injected arginine vasopressin was thoroughly mixed with repeated withdrawals and reinjections of amniotic fluid. In a second group of four fetuses, 18 ml of warmed normal saline solution containing 22.5 µg of arginine vasopressin was injected into the catheter that was sewn into the glove over the fetal head, followed by 5 ml of normal saline solution. In one animal from each group the opposite protocol (glove or amniotic fluid injection) was performed on the animal 4 days later. Fetal and maternal arterial plasma and amniotic fluid were sampled at 10 and 20 minutes during the 30-minute control period and at 15, 30, and 60 minutes after the injection of arginine vasopressin. A 2 ml sample of the injection solution from both groups was assayed for arginine vasopressin concentration. Fetal arterial blood (from the descending aorta) was sampled for blood gases and pH (Instrumentation Laboratory [Lexington, Mass.] model 1302 blood gas analyzer); sodium, chloride, and potassium ion concentrations (Nova Model 5 + 5 electrolyte analyzer, Waltham, Mass.); osmolality (freezing point depression, Advanced Instruments Inc., Needham Heights, Mass.); hematocrit; and plasma concentration of arginine vasopressin. Maternal blood and amniotic fluid were sampled for electrolytes and osmolality at the same times as stated above. The amniotic fluid volume was estimated in the intraamniotically injected group by dividing the amount of arginine vasopressin injected by the mean amniotic arginine vasopressin concentration during the 1 hour after the injection.

Radioimmunoassay. Plasma and amniotic samples were extracted with C₁₈ microcolumns (Sep-Pak, Waters Associates, Milford, Mass.), and arginine vasopressin concentrations were measured by radioimmunoassay with an antiserum generously provided by Dr. Ben U (University of California, San Diego) and tracer obtained from New England Nuclear (Boston), as previously described.5 Extraction recovery averaged $84\% \pm 3\%$, and sensitivity was 0.2 pg per tube. The intraassay and interassay coefficients of variation were 6% and 14%, respectively.

Statistical analysis. The data are presented as the mean ± SE. Changes with time in a single variable were analyzed with a two-factor repeated-measures analysis of variance, with time and animal being the two factors. All parameters from the control period of each group were compared with group t tests. All arginine vasopressin concentrations (plasma and amniotic fluid samples) were log transformed before analysis to correct for skewness of data. To determine whether changes in time in one variable were significantly different when the two groups of animals were compared, a threefactor repeated-measures analysis of variance was used, with time, treatment, and animal being the factors. Statistical significance was accepted at $p \le 0.05$, and changes referred to as significant are at this level unless specified otherwise.

At the end of the experiments, the animal was killed

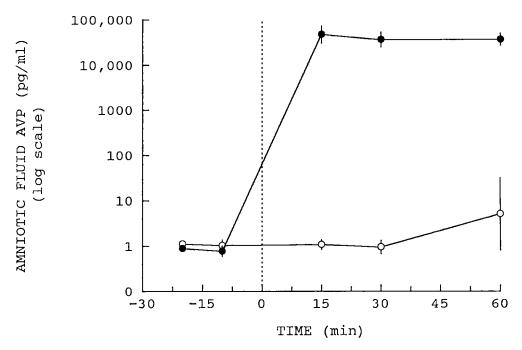


Fig. 1. Mean (±SEM) amniotic fluid arginine vasopressin concentrations (log scale) after injection of 22.5 µg arginine vasopressin (dotted vertical line) into amniotic cavity (closed circles) or gloved fetal head (open circles). Changes from control period are highly significant (p < 0.00001) for intraamniotically injected group, but no significant change occurred in glove-injected animals.

Table I. Mean (±SE) control values before arginine vasopressin injection*

	Site of injection of 22.5 µg of arginine vasopressin				
Variable	Amniotic fluid	Glove			
Fetus		-			
Gestational age (days)	132 ± 2	130 ± 1			
Arterial pH	7.33 ± 0.01	7.32 ± 0.01			
Pco ₂ (mm Hg)	55.8 ± 1.4	55.5 ± 0.6			
Po ₂ (mm Hg)	19.0 ± 1.6	21.0 ± 0.8			
Hematocrit (%)	34.2 ± 2.0	36.7 ± 2.7			
Weight (kg)	3.5 ± 0.2	3.0 ± 0.4			
Vasopressin (pg/ml)					
Fetal plasma	4.5 ± 1.3	5.5 ± 1.3			
Amniotic fluid	6.1 ± 0.9	7.6 ± 1.5			
Maternal plasma	1.5 ± 0.2	1.2 ± 0.04			

^{*}In each group n = 5 animals except n = 4 in arginine vasopressin measurements in glove-injected group. There were no statistical differences between groups in the abovemeasured parameters.

with pentobarbital sodium (300 mg/kg), an autopsy was performed on the fetus, and fetal weight was recorded.

Results

Mean values during the control period for the two groups of animals are shown in Table I. No statistically significant differences were found between either group, and these control values are consistent with those values found previously in our laboratory. Fig. 1

shows the arginine vasopressin concentrations in the amniotic fluid before and after the injection of 22.5 µg arginine vasopressin into either the amniotic cavity or the glove covering the fetal head. Amniotic fluid concentrations increased sharply after injection of arginine vasopressin into the amniotic cavity, and the concentration remained elevated for the duration of the experiment. Although the changes with time in concentrations were highly significant (p < 0.00001) for the intraamniotically injected group, there was no significant change over time in the amniotic fluid concentration when the arginine vasopressin was injected into the glove over the fetal head. However, in the latter group there was a nonsignificant increase in the amniotic fluid arginine vasopressin concentration at 60 minutes after the injection, as a result of a rise in one animal.

The fetal plasma arginine vasopressin concentrations are presented in Fig. 2. Significant increases (p < 0.00001) were observed in the amniotically injected group within 15 minutes, and a maximal level was reached within 60 minutes. There were small increases over time in fetal plasma arginine vasopressin concentrations after injection into the glove, but this did not reach statistical significance. This increase in the gloved group was on average 6.8% of that which occurred after intraamniotic injection. In several animals in both groups, fetal plasma arginine vasopressin samples were taken 2 hours after the injection. In the

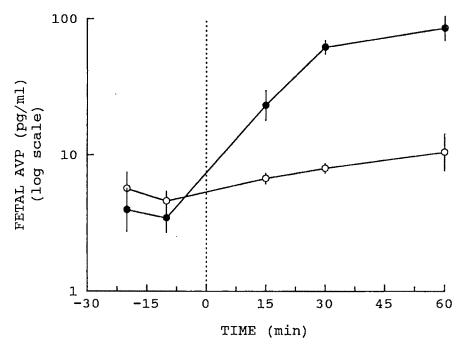


Fig. 2. Mean (±SEM) fetal arterial plasma arginine vasopressin concentration (log scale) after injection of 22.5 µg arginine vasopressin (dotted vertical line) into amniotic cavity (closed circles) or gloved head (open circles). Intraamniotic group had significant increases within 15 minutes when compared with both control period and glove-injected group (p < 0.00001). There were no significant changes in glove-injected group over time.

amniotically injected group, the concentration did not change from the 1-hour postinjection value. In the intraglove-injected group, there was a further nonsignificant increase in arginine vasopressin concentration. In one animal in the gloved group, 22.5 µg was injected into the amniotic cavity 1 hour after the injection into the glove over the fetal head. In that animal there were large increases in amniotic fluid and fetal plasma arginine vasopressin concentrations and arterial pressure and decreases in heart rate.

Fig. 3 shows the changes in fetal arterial pressure and heart rate after the injection of arginine vasopressin. Significant increases in arterial pressure of 10 \pm 2 mm Hg (p < 0.00001) and decreases in heart rate of 30 ± 5 beats/min occurred in the intraamniotically injected group. These increases in the arterial pressure occurred within 15 minutes after the intraamniotic injection, with significant decreases in heart rate by 20 minutes after the injection. These physiologic changes in arterial pressure and heart rate coincide with the measured increase in fetal plasma arginine vasopressin concentrations (Fig. 2). There were no significant changes in either arterial pressure or heart rate in the group with the arginine vasopressin injected into the glove over the fetal head. There were no significant changes in either the venous pressure or the amniotic pressure after the injections in either group.

There were no changes over time in either group

with respect to fetal pH, Pco₂, Po₂, plasma osmolality, or electrolyte concentrations. Likewise, there were no differences between groups over time with respect to amniotic fluid osmolality (during control period, 286 ± 8 mOsm/kg for amniotic fluid-injected group and 292 ± 5 mOsm/kg for the glove-injected group). The maternal plasma osmolalities (during control period 303 ± 4 mOsm/kg for the amniotic fluid-injected group and 304 ± 2 mOsm/kg for the glove-injected group) were different neither over time nor between groups. The maternal arginine vasopressin concentrations did not change significantly over time and averaged 2.2 ± 0.6 pg/ml for the amniotic fluid-injected group and 1.6 ± 0.5 pg/ml for the glove-injected group after the injection.

The amniotic fluid volume in the intraamniotically injected group was calculated to be 569 ± 143 ml (range, 174 to 933 ml). This value is consistent with volumes determined previously in our laboratory.

At autopsy, the surgical glove remained sutured over the fetal head, and the catheter was in place within the glove in all animals. The glove was neither overly distended nor collapsed but contained a modest amount of fluid.

Comment

In this study we found minimal absorption of arginine vasopressin into the fetal circulation when it was

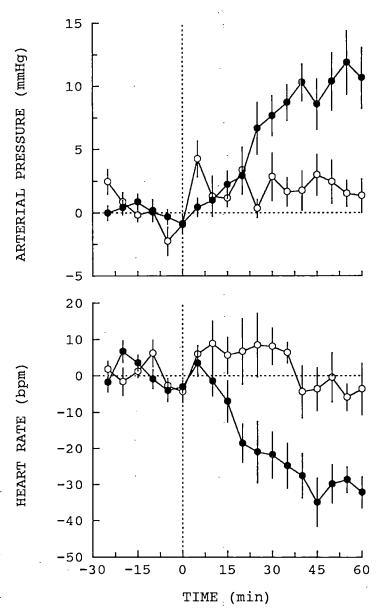


Fig. 3. Mean (\pm SEM) change from control in fetal arterial pressure (*upper*) and heart rate (*lower*) after injection of 22.5 µg arginine vasopressin (*dotted vertical line*) into amniotic cavity (*closed circles*) and gloved head (*open circles*). Arterial pressure increased significantly in intraamniotically injected group (p < 0.00001) but was unchanged in glove-injected group. Fetal heart rate decreased significantly in intraamniotically injected group (p < 0.00001) and was unchanged in glove-injected group.

injected into a glove covering the fetal head. Conversely, we found a rapid and significant absorption into the fetal circulation when it was injected into the amniotic cavity when a glove covered the fetal head. From this we conclude that the fetal oral-nasal membranes are not a significant route for the absorption of amniotic fluid arginine vasopressin relative to that which occurs across the fetal surface of the placenta and the vascularized fetal membranes (i.e., the intramembranous pathway). We did find, however, a small

increase in fetal plasma arginine vasopressin concentrations in three of four animals when it was injected into the glove covering the fetal head. This increase in concentration was minor (6.8%) when compared with the amount absorbed through the intramembranous pathway. This increase would suggest that a small amount of arginine vasopressin may be absorbed by the oral-nasal membranes or fetal swallowing.

In adult humans the nose is highly vascularized and is used to administer certain medications such as vasopressin in patients with diabetes insipidus. Furthermore, sublingual nitroglycerin is rapidly absorbed in subjects with cardiovascular disease. Thus, in spite of the presence of this route of absorption in the adult, it would appear that the fetal oral-nasal membranes are not a major route for absorption in utero relative to the absorption that occurs through the intramembranous pathway.

Another point to strengthen this conclusion is that we injected 22.5 µg of arginine vasopressin into a closed space over the fetal head in one of the groups of fetuses. Thus arginine vasopressin concentrations within the glove would be very high because of the small volume of distribution. In the second group we injected 22.5 µg of arginine vasopressin into the amniotic cavity, which has a much larger volume of distribution. Therefore the fetal oral-nasal membranes most likely were exposed to a much higher concentration than was the intramembranous pathway. This difference in concentrations strengthens our conclusion that oral-nasal absorption of arginine vasopressin appears small compared with intramembranous absorption.

In our prior study² we injected 1 to 25 µg of arginine vasopressin into the amniotic cavity and found that the absorbed arginine vasopressin was physiologically active. In this study we found similar results, in that there was a rapid absorption of physiologically active arginine vasopressin as manifested by an increase in arterial pressure and a fall in heart rate (Fig. 3). Ervin et al.6 had previously injected 25 µg of arginine vasopressin into the ovine amniotic cavity and found that the fetal plasma concentrations had increased within 30 minutes. They found that this increase, which they felt was due to fetal swallowing, did not significantly change any physiologic variable such as heart rate or arterial pressure. Our study demonstrated that the arginine vasopressin absorbed into the fetal circulation was not absorbed primarily by fetal swallowing. Near the end of the study, in the glove-injected group there was a nonsignificant increase in amniotic fluid and fetal plasma arginine vasopressin concentrations. This may indicate a small amount of absorption by the oral-nasal pathway, absorption by fetal swallowing, or spillage of arginine vasopressin from the neck of the glove into the amniotic cavity with subsequent intramembranous absorption. However, this absorption was minimal compared with the absorption after intraamniotic injection. Since neither fetal swallowing nor the oral-nasal membranes appear to be a major pathway for the absorption of amniotic fluid arginine vasopressin, the only remaining route for its movement into the fetal circulation is the intramembranous pathway.

Although this study confirms our previous stud-

ies,1,2 we are unable to explain the finding of Hamamoto et al.8 of a higher level of digoxin in the descending aorta when compared with that in the umbilical vein. A possible explanation is that the fetal oral-nasal membranes may absorb digoxin but not arginine vasopressin much more rapidly than does the intramembranous pathway. This difference cannot be resolved until the absorption of intraamniotic digoxin in fetuses is studied in more detail.

It may be suggested that a method to evaluate the extent of arginine vasopressin absorption by the intramembranous pathway would be to examine the umbilical arterial-venous differences. There are at least three potential problems with this idea. One is that arginine vasopressin is cleared by the placenta.7 The second is that the umbilical blood flow rate is large, with a flow of 200 ml/min/kg fetal body weight.8 In our fetuses (3.25 kg) umbilical blood flow would equal 650 ml/min through the umbilical vein. The third is that only a moderate to large arginine vasopressin concentration difference could be detected because of inherent assay variability in combination with the high umbilical blood flow rate plus the placental metabolism of arginine vasopressin. Thus, because fetal plasma arginine vasopressin concentrations increased only slowly over 1 hour, an arterial-venous concentration difference would most likely not be detectable in the present study.

At autopsy we found that the glove over the fetal head contained a modest amount of fluid. Previously, in a group of ovine fetuses who had both the trachea and the esophagus ligated we found that a glove sutured in place over the fetal head was distended with thick, tenacious secretions at autopsy.9 Thus this study would imply that the fetus continued to swallow its oral and lung secretions in spite of the glove over the its head. Whether the glove affected breathing movements is unknown.

In summary, this study shows that intraamniotically injected arginine vasopressin is rapidly absorbed by the fetal blood that perfuses the fetal surface of the placenta and the fetal blood perfusing the membranes. We would speculate that this intramembranous pathway may provide an avenue by which we can treat fetal disease by administering medications such as digoxin into the amniotic cavity to treat fetal cardiac arrhythmias or antibiotics to treat fetal infections.

We thank R. Scot Payne and Tamara Tetzke for their assistance with this study.

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Amniotic fluid volume response to esophageal occlusion in fetal sheep

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Although ovine and human fetuses swallow considerable volumes of fluid, the impact of absence of fetal swallowing on amniotic fluid volume regulation is unclear. To study the role of fetal swallowing on urine production and amniotic fluid, seven ovine fetuses (126 ± 1 days) were chronically prepared with fetal biadder and vascular catheters, an esophageal flow probe, an inflatable esophageal cuff, and amniotic fluid catheters. In the five fetuses that underwent esophageal ligation after the control period, fetal swallowing averaged 0.27 ml/min before occlusion. In response to esophageal occlusion, significant increases were noted in fetal plasma arginine vasopressin (6.9 ± 2.6 to 16.6 ± 4.4 pg/ml) and urine osmolality (159 ± 1 to 324 ± 30 mOsm/kg), whereas urine volume (0.25 ml/min) did not change. Amniotic fluid volume increased nearly threefold after 3 days of esophageal occlusion (582 ± 180 to 1530 ± 271 ml). Amniotic fluid volume remained normal (334 to 419 ml) in the one fetus in which the occluder did not inflate. In the one fetus in which the esophagus was occluded at surgery, amniotic fluid volume was increased after the surgical recovery period (1489 ml). These data indicate an important role of fetal swallowing in amniotic fluid homeostasis and the potential interaction of swallowing with fetal urine production. (Am J Obster Gynecol. 1991;165:1620-6.)

Key words: Ovine fetus, fluid balance, polyhydramnios

Amniotic fluid volume homeostasis results from a balance of fetal fluid secretion and resorption. In the near-term ovine pregnancy, fetal urine contributes from 400 to 1200 ml per day to the amniotic or allantoic cavities. Although the fetal lung produces 200 to 400 ml of fluid per day, a major portion of ovine fetal lung

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fluid is swallowed.^{2,3} In addition to ingested lung fluid, ovine and human fetuses may swallow up to 1000 ml of amniotic fluid per day.^{2,4-7} Amniotic fluid water and electrolytes also may be exchanged by an intramembranous route to the fetus (across the amniotic membranes through intramembrane vessels⁸) or a transmembranous route to the mother.⁹

Congenital anomalies that impair or prevent human fetal swallowing (anencephaly, esophageal atresia, myotonic dystrophies) are frequently associated with polyhydramnios, confirming a role of swallowing in amniotic fluid regulation.¹⁰ However, polyhydramnios does not invariably develop in these pregnancies.¹¹

The role of fetal swallowing in the regulation of amniotic fluid volume has been investigated in several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid volume has been investigated in the several anniotic fluid

imal models. Esophageal ligation of the fetal rhesus monkey resulted in transient polyhydramnios, with amniotic fluid volume returning to normal within 2 weeks.12 In studies of the ovine fetus amniotic fluid volumes 3 weeks after acute surgical esophageal ligation were not different from control values.13 The authors concluded that deglutition is not necessary for the regulation of amniotic fluid volume in sheep.13 However, acute effects of esophageal ligation were not examined, nor was fetal urine or lung fluid production measured to determine if compensatory adaptations occurred. Similarly, the acute urinary effects of esophageal ligation have not been examined, although Smith et al.14 reported normal fetal urinary volume and osmolality after recovery from surgical ligation of the esophagus.

In this study we evaluated the effects of esophageal occlusion on fetal urine production and amniotic fluid volume.

Material and methods

Animals and surgery. Seven Western crossbred pregnant sheep with singleton fetuses were studied at a mean gestational age of 126 ± 1 day. Guidelines approved by Harbor-University of California Los Angeles Medical Center for the care and use of animals were followed. Animals were housed indoors in individual steel study cages and maintained under a controlled 12-hour-light/12-hour-dark lighting regimen. Both food and water were available at will, except for the withholding of food during the 24-hour period immediately before surgery.

Anesthesia for surgery was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg) plus atropine sulfate (30 µg/kg) and subsequently maintained by a continuous intravenous infusion of ketamine (15 mg/kg/hour). The uterus was exposed by a midline abdominal incision, and a small hysterotomy was performed to provide access to a single fetal hind limb. Polyethylene catheters (1.0 mm inner diameter, 1.8 mm outer diameter) were placed in the fetal dorsal hind limb vein and artery and threaded to the inferior vena cava and abdominal aorta, respectively. Before hysterotomy closure, a plastic catheter was sutured to the distal tip of the hind limb and a second catheter was placed adjacent to the fetal head to provide access to the amniotic cavity. The hind limb was returned into the uterus, and the hysterotomy was closed in two layers.

A second hysterotomy was performed, and a polyethylene catheter was placed in the fetal bladder by cystotomy. Through a third hysterotomy the fetal left hemithorax was entered through the eighth intercostal space. The peritoneum overlying the esophagus was incised for a distance of 3 cm cephalad to the diaphragm.

An inflatable occluder (In Vivo Metric Systems, Healdburg, Calif.) was placed on the thoracic esophagus, and an ultrasonographic flow probe (Transonic System Inc., Ithaca, N.Y.) was placed around the esophagus, caudal to the occluder. The fetal chest was closed in layers, the uterine and maternal incisions were repaired, and catheters were externally accessed through a maternal flank incision. Catheters also were placed in the maternal inferior vena cava and abdominal aorta through the femoral vein and artery in one leg.

Fetal vascular catheters were maintained patent by filling the catheter dead space (I ml) with heparin sodium (1000 U/ml); catheters were sealed with sterile plastic caps. Maternal vascular catheters were filled with heparinized saline solution (10 U/ml), and both fetal and maternal catheters were flushed daily with heparinized saline solution (10 U/ml).

All animals were allowed a minimum of 5 days for postoperative recovery, during which time the fetuses received intravenous infusions of oxacillin (33 mg) and gentamicin sulfate (8 mg) twice daily for 2 days. Oxacillin (967 mg), chloramphenicol (500 mg), and gentamicin sulfate (72 mg) also were administered into the maternal vein.

Study design. The study design consisted of 4 days of fetal assessment. On each of the study days fetal urine production, esophageal flow, and fetal and maternal arterial blood pressures and amniotic fluid pressure were monitored. Amniotic fluid volume determinations were performed on days 1 and 4.

Study day 1 was performed as follows: The fetal bladder catheter was opened to gravity and drained during a 1-hour equilibration period. Beginning at time 0, fetal urine flow was collected at 15-minute intervals during a 1-hour control period (time 0 to 1 hour) while fetal esophageal flow was measured continuously. At time 30 minutes and 1 hour, fetal, maternal, and amniotic fluid pressures were measured, and fetal and maternal arterial blood samples (4 ml) were withdrawn. Fetal blood samples were replaced with an equivalent volume of maternal blood drawn before the study, and maternal blood samples were replaced with an equivalent volume of 0.9% saline solution. Hematocrit, pH, PO2, Pco₂, plasma osmolality, and sodium, chloride, potassium, and arginine vasopressin concentrations were measured.

At time 1 hour, amniotic fluid aliquots (1 ml) were obtained for background counts and technetium 99labeled maternal red blood cells were injected into the amniotic fluid. Amniotic fluid was mixed by gentle shaking of the maternal abdomen with an external sling.15 Amniotic fluid samples were withdrawn from both amniotic catheters for 6 hours (at time 2 hours and from time 3 to 7 hours at 30-minute intervals). Fetal urine production was monitored for an additional 2 hours after the injection of ⁹⁹Tc-labeled red blood cells and urine volume, osmolality, and electrolytes were measured. All fetal urine samples were returned to the amniotic cavity at hourly intervals. Fetal swallowing was measured for 7 hours (time 0 to 7 hours). Repeat fetal and maternal blood samples were drawn at time 3 and 7 hours.

At time 7 hours of study day 1, the fetal esophagus was occluded with the inflatable occluder, and the absence of flow was confirmed with the flowmeter.

On study days 2 and 3, after a 1-hour equilibration period, fetal urine was drained into sterile tubes for 1 hour. The esophageal occluder was emptied of fluid and reinflated each morning to assure full inflation. Esophageal flow was monitored to confirm the absence of flow. Fetal and maternal blood samples were withdrawn at time 0 and 1 hour.

Study day 4 was identical to day 1 with the exception that the esophagus remained occluded for the duration of the study.

Analytic methods

Measurements of amniotic fluid volume. The volume of amniotic fluid was measured with ^{99}Tc -labeled red blood cells. Maternal red blood cells were labeled with a kit (Cameda Medical Products Inc., Md.) modified by the addition of 0.6 ml of 0.1% sodium hypochlorite. After the ^{99}TC (400 μCi) addition, an aliquot of the maternal red blood cell solution was counted and the injected dose calculated by weighing the syringe before and after injection.

Amniotic fluid samples were counted for ⁹⁹Tc on a 1282 Compugamma γ-counter (LKB Wallac, Turku, Finland) incorporating a 6-hour half-life correction. Amniotic fluid volume was calculated by extrapolation to time 0 of the semilogarithmic regression line of the average ⁹⁹Tc counts (withdrawn from both amniotic catheters) versus time, as described previously.^{7, 15}

Maternal and fetal blood pressures and heart rate were monitored with a Beckman R-612 physiologic recorder (Beckman, Palo Alto, Calif.) and Statham P-23 transducers. Mean fetal blood pressure was corrected for amniotic fluid pressure. Maternal and fetal blood pH, PO₂, and PCO₂ values were determined at 39° C with a Radiometer BMS2 MK2 acid-base analyzer system (Radiometer CO., Copenhagen). Plasma osmolality was measured by freezing point depression on an advanced digimatic osmometer (Model 3MO, Advanced Instruments, Needham Heights, Mass.). Plasma, urine, and amniotic fluid electrolyte levels were determined by a NOVA5 electrolyte analyzer (NOVA Biochemical, Waltham, Mass.). Plasma arginine vasopressin levels were measured by radioimmunoassay of plasma (1 ml) extracted on Sep-Pak columns (Waters Associates, Milford, Mass.), as previously described from our laboratory.16

Fetal volume swallowed was calculated as positive flow minus negative flow with a transit-time ultrason-ographic flowmeter and computer analysis, as previously described from our laboratory. ^{5, 6} The data are presented as volume swallowed per unit time.

Urinary sodium excretion was expressed as the product of the urine sodium concentration and urine volume. Urinary potassium, chloride, and osmolar excretions were calculated in the same manner. Renal osmolar clearance (Cosm) was calculated as Cosm (ml/min) = (UosmV)/Posm, where UosmV is urinary osmolar excretion and Posm is plasma osmolality. Renal free water clearance (CH₂O) was calculated as the difference between the urine volume and the renal osmolar clearance: $CH_2O = Uvol - Cosm$.

Statistics and data analysis. All values are expressed as the mean \pm SEM. Mean values for study periods (before and after ⁹⁹Tc-labeled red blood cells injection) and study days were determined. Differences between study periods were assessed by repeated-measures analysis of variance with trends in the data considered statistically significant when the F test yielded a p < 0.05. Comparisons of the control values to the study periods were determined with Dunnett's test (p < 0.05). Linear and polynomial regression were used to evaluate the relationship of fetal plasma arginine vasopressin and blood pressure and urinary parameters. Differences between amniotic fluid values were determined by paired t test.

Results

Five of the seven animals surgically prepared completed the study as intended. The results are presented for these five fetuses. In one fetus the esophageal occluder did not inflate and in one fetus the esophagus was inadvertently occluded at the time of surgery. The amniotic fluid volume responses in these two fetuses are presented for comparison.

Fetal arterial values. There was no significant change in fetal arterial pH, PCO_2 , hematocrit, heart rate, plasma osmolality, or electrolyte composition in response to esophageal occlusion. There was a near-significant trend toward a decrease in fetal arterial PO_2 during the study (p=0.057) (Table I). Fetal mean blood pressure (43 ± 2 to 46 ± 3 mm Hg), plasma potassium level (3.9 ± 0.1 to 4.5 ± 0.1 mEq/L), and plasma arginine vasopressin level (6.9 ± 2.6 to 16.6 ± 4.4 pg/ml) increased significantly on study day 4 (Table I, Fig. 1). Fetal plasma arginine vasopressin level was significantly correlated with changes in fetal blood pressure (p=0.009, p=0.602).

Maternal arterial values. Maternal pH (7.46 ± 0.02) , Po₂ $(86.1 \pm 0.8 \text{ mm Hg})$, Pco₂ $(32.1 \pm 3.0 \text{ mm Hg})$, hematocrit $(31.5\% \pm 1.6\%)$, mean blood pressure $(95 \pm 6 \text{ mm Hg})$, heart rate $(125 \pm 4 \text{ beats/min})$,

				, 1 0		
	Day 1				Day 4	
	Control	After injection	Day 2	Day 3	Control	After injection
pH	7.37 ± 0.01	7.37 ± 0.01	7.36 ± 0.01	7.36 ± 0.01	7.36 ± 0.02	7.36 ± 0.02
PO ₂ (mm Hg)	24.8 ± 1.7	21.3 ± 1.3	21.2 ± 2.7	18.9 ± 2.3	18.3 ± 3.0	21.3 ± 2.0
PCO ₂ (mm Hg)	42.0 ± 2.5	41.9 ± 2.6	44.9 ± 4.1	43.9 ± 2.5	44.6 ± 3.4	45.3 ± 2.4
Hematocrit (%)	32.7 ± 1.7	32.1 ± 1.8	31.9 ± 1.9	3.4 ± 2.0	31.7 ± 2.1	31.4 ± 2.2
Blood pressure (mean, mm Hg)	43 ± 2	43 ± 2	42 ± 2	44 ± 2	47 ± 3*	46 ± 3*
Heart rate (beats/min)	172 ± 3	170 ± 4	175 ± 3	171 ± 5	172 ± 8	174 ± 10
Osmolality (mOsm/kg)	293 ± 2	292 ± 3	293 ± 3	294 ± 3	292 ± 3	295 ± 3
Sodium (mEq/L)	142.7 ± 0.4	142.9 ± 0.5	143.1 ± 0.1	143.9 ± 1.3	143.8 ± 2.0	143.2 ± 1.3
Potassium (mEq/L)	3.9 ± 0.1	3.9 ± 0.1	4.1 ± 0.1	$4.3 \pm 0.1*$	$4.5 \pm 0.2*$	4.5 ± 0.1 *
Chloride (mEq/L)	110.9 ± 1.3	111.0 ± 1.1	110.3 ± 0.6	109.2 ± 0.6	109.1 ± 1.7	108.8 ± 1.2

Table I. Fetal arterial values before (day 1) and after (days 2, 3, and 4) exophageal occlusion

plasma osmolality (298 ± 2 mOsm/kg), and plasma arginine vasopressin (6.1 \pm 2.7 pg/ml), sodium $(149.9 \pm 0.6 \text{ mEq/L})$, potassium $(4.3 \pm 0.2 \text{ mEq/L})$, or chloride (112.9 \pm 0.6 mEq/L) concentrations did not change.

Fetal urine values. Fetal urine volume did not change from basal values of 0.25 ± 0.04 ml/min during the study (Fig. 1). However, fetal urine sodium, potassium, and chloride concentrations and osmolality significantly increased in response to esophageal occlusion (Table II, Fig. 1). Consequently, urinary sodium, potassium, chloride, and osmolar excretions significantly increased and free water clearance decreased. Fetal urinary osmolar excretion (p = 0.04, r = 0.514) and osmolar clearance (p = 0.022, r = 0.553) were significantly correlated with fetal plasma arginine vasopressin level. However, there was not a significant correlation of free water clearance and arginine vasopressin level (p = 0.068, r = 0.475).

Fetal swallowing and amniotic fluid. Fetal swallowing averaged 0.27 ± 0.07 ml/min before esophageal occlusion. Basal amniotic fluid volume (582 ± 180 ml) significantly increased on study day 4 after esophageal occlusion (1530 \pm 271 ml) (Fig. 1). There were no changes in amniotic fluid osmolality or electrolyte composition (Table III).

In the fetus in which the esophageal occluder did not inflate, amniotic fluid volume was 334 ml on study day 1 and 419 ml on study day 4. Fetal swallowing averaged 0.16 and 0.25 ml/min, respectively. In the fetus in which the esophagus was occluded at surgery, esophageal flow was not detected during the study. Amniotic fluid volumes on day 1 and 4 were 1489 and 1530 ml, respectively.

Comment

In all fetuses in this study normal physiologic parameters were demonstrated during the control period before esophageal occlusion. Mean basal fetal urine osmolality (159 mOsm/kg), a sensitive index of fetal stress, was in the normal range for fetuses of this gestational age. In spite of the normal urine osmolality, the basal urine production rate (0.1 ml/kg/min) was below previously reported values from our laboratory and others.17-19 Notably, Daniel et al.20 have demonstrated a wice range of normal fetal urine flow rates (0.1 to 0.4 ml/kg/min) with urine osmolalities <200 mOsm/kg. Similarly, Gresham et al.1 noted "large interindividual differences in urine flow (0.09 to 0.31 mg/kg/min) with urine osmolalities <160 mOsm/kg. Thus biologic variability may have contributed in part to the low Lrine production rates in our study. Numerous other factors, including atrial natriuretic factor, catecholamines, and aldosterone, may alter fetal urine production or composition. Basal fetal urinary sodium concentration was 25% to 40% less than that noted in previous studies by our laboratory and others. 17-19 Although fetal plasma atrial natriuretic factor and maternal-fetal aldosterone were not measured in our study, it also is possible that alterations in these values contributed to the reduced urine volume.

Amniotic fluid volume (582 ml) and osmolality (275 ± 3 mOsm/kg) during the control period were consistent with previous determinations.7, 15, 15, 21 Thus there was no indication that the surgical preparation influenced basal amniotic fluid volume. Fetal volume swallowed before esophageal occlusion (0.27 ml/min) extrapolated to 392 ml/day, a volume similar to the extrapolated daily urine volume.

In response to esophageal occlusion, fetal plasma arginine vasopressin and urine osmolality significantly increased, although urine flow rates did not change. With the exception of a slight increase in plasma potassium, there were not statistically significant changes in fetal arterial values on days 2 or 3. Although fetal plasma arginine vasopressin was not statistically greater than basal levels until day 4, plasma arginine vasopressin levels similar to those observed on days 2 and 3 may

^{*}p < 0.05, versus day 1 control.

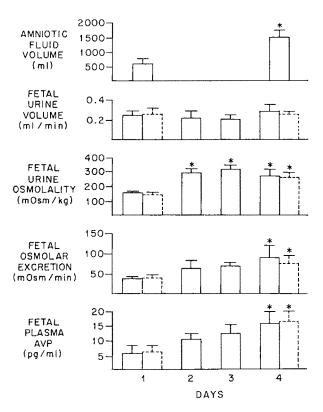


Fig. 1. Amniotic fluid volume and fetal urine and plasma arginine vasopressin values before (day 1) and after (days 2, 3, and 4) esophageal occlusion. Bars depicted in dashed lines are values after intraamniotic injection and mixing of 99 Tc. Asterisk, p < 0.05, versus day 1 values.

induce production of hypertonic urine in fetuses >110 days' gestation.²² Increased fetal plasma arginine vasopressin generally is associated with a reduced urinary flow rate.²⁰ However, Daniel et al.²⁰ demonstrated significant increases in fetal urine osmolality with minimal volume changes at lower levels of urine flow rates. Thus the maintenance of urinary flow rates and the lack of a statistically significant correlation between plasma arginine vasopressin and fetal urinary free water clearance suggests that esophageal occlusion affected other regulatory factors in addition to arginine vasopressin.

Fetal mean arterial blood pressure significantly increased on study day 4 in association with plasma arginine vasopressin levels of 16 pg/ml. Although Tomita et al. ²² demonstrated no increase in fetal arterial blood pressure with a 6.5 \pm 3.9 pg/ml increase in plasma arginine vasopressin, Wiriyathian et al. ²³ demonstrated increased fetal mean arterial pressure at plasma arginine vasopressin levels of 8 to 9 μ U/ml (18 to 20 pg/ml). In the present study, the significant correlation between fetal plasma arginine vasopressin and arterial blood pressure suggests that the elevated plasma levels noted on day 4 contributed to the increased fetal blood pressure.

The increase in plasma arginine vasopressin suggests that the fetus attempted to conserve water that was no longer available by gastrointestinal resorption. Although the mechanism for the increased levels is uncertain, it may have contributed to the maintenance of normal fetal plasma osmolality and hematocrit. There were no acute blood pressure or heart rate changes indicative of aortic constriction or cardiovascular compromise. Although fetal venous and lymphatic pressures were not determined, it appears that baroreceptor responses did not contribute to increased arginine vasopressin secretion. Notably, the occluder was placed between the esophagus and the vagus nerve, so as not to alter vagal innervation. Compression of the esophagus and potential esophageal distention (with fluid sequestration proximal to the occluder) may have induced a nonspecific fetal arginine vasopressin stress response. Although not statistically significant, the tendency toward reduced fetal arterial Po2 also may have potentiated arginine vasopressin stimulation.24 Neither Wintour et al.18 nor Smith et al.14 reported plasma arginine vasopressin levels after acute surgical ligation. However, Smith et al.14 reported urinary osmolality/plasma osmolality ratios of 0.42 to 0.69 at 5 days after surgery, suggesting the absence of an arginine vasopressin response. Nevertheless, the lack of measurements during the initial 4 days after esophageal occlusion limits comparisons to the acute effects of esophageal occlusion in the present study.

Amniotic fluid volume increased nearly threefold after esophageal occlusion. In sheep, amniotic fluid is produced by fetal urine production and lung liquid that is not swallowed. Amniotic fluid resorption occurs by fetal swallowing and perhaps transmembraneous or intramembranous flow.8 After esophageal occlusion in the present study, fetal urine production continued at an equivalent rate, presumably contributing to amniotic fluid volume. Although within normal limits (as discussed above), the relatively low rates of urine flow throughout the study likely would have moderated the increase in amniotic fluid volume. Nevertheless, continued urine production indicates that fetal swallowing and urine flow rates may not be coupled under pathologic conditions. Fetal lung fluid, although not measured, was likely secreted into the amniotic cavity rather than swallowed. Thus there was continued urine and likely increased lung fluid contribution to the amniotic fluid. With fetal swallowing prevented, increased amniotic fluid volume would be expected. Notably, a 3day extrapolation of the control period volume swallowed (1196 ml) is similar to the measured increase in amniotic fluid volume (948 ml).

Although a control series of fetuses was not studied, Lingwood and Wintour²¹ noted amniotic fluid volume increases average <10 ml per day between 120 and

Table II. Fetal urine values before (day 1) and after (days 2, 3, and 4) esophageal occlusion

<u> </u>	Before o	occlusion		After occ	lusion	
	Day 1 (after control)	Day 1 (after injection)	Day 2	Day 5	Day 4 (control)	Day 4 (injection)
Sodium (mEq/L)	32.2 ± 4.2	31.6 ± 1.5	79.6 ± 19.7*	90.1 ± 17.2*	84.7 ± 16.7*	69.8 ± 15.2*
Potassium (mEq/L)	7.31 ± 4.21	9.90 ± 5.30	12.47 ± 5.24	$20.84 \pm 3.25*$	$32.00 \pm 6.50*$	31.82 ± 8.55*
Chloride (mEq/L)	26.6 ± 3.0	31.3 ± 5.9	$65.1 \pm 20.3*$	$82.6 \pm 20.1*$	92.0 ± 22.2*	79.2 ± 23.5*
Sodium ex- cretion (µEq/min)	9.26 ± 1.33	7.13 ± 0.84	17.37 ± 4.76*	18.92 ± 4.19*	20.64 ± 3.60*	17.44 ± 2.16*
Potassium excretion (µEq/min)	2.42 ± 0.71	3.60 ± 0.98	3.20 ± 1.16	7.28 ± 0.23*	8.86 ± 1.79*	9.15 ± 1.51*
Chloride ex- cretion (µEq/min)	7.92 ± 0.92	8.86 ± 1.58	15.48 ± 5.07	17.34 ± 4.23*	23.85 ± 4.52*	20.29 ± 3.27*
Osmolar clearance (ml/min)	0.13 ± 0.02	0.13 ± 0.03	0.22 ± 0.06	0.23 ± 0.04	$0.30 \pm 0.09*$	0.25 ± 0.06*
Free water clearance (ml/min)	0.118 ± 0.058	0.130 ± 0.029	$-0.001 \pm 0.019*$	-0.042 ± 0.025*	-0.013 ± 0.026*	0.010 ± 0.028*

^{*}p < 0.05, versus day I control.

130 days' gestation and Tomoda et al.25 noted that amniotic fluid volume changed only "slightly from day to day." Furthermore, amniotic fluid volume did not appreciably increase in the single animal studied without esophageal occlusion. Thus the marked increased in amniotic fluid volume is likely a result of the esophageal occlusion and absent swallowing.

Hutchinson et al.,9 using isotope injections into the amniotic cavities of women, suggested that polyhydramnios was associated with differences in the rates of reciprocal transfer between mother, fetus, and amniotic fluid; however, these differences served to maintain an overall balance. Notably, this conclusion was derived from patients already diagnosed with polyhydramnios, whereas our study acutely induced polyhydramnios. Thus, in contrast to the present study, Hutchinson et al.9 did not observe the initial imbalance in fluid exchange resulting in polyhydramnios. The stable, though increased, amniotic volume, in the ovine fetus in which the esophagus was occluded at surgery, suggests that after the acute imbalance an equilibrium of transfer rates may occur. Although these findings concur, it is noteworthy that the diffusionary water flow measured by isotope techniques9 differs from the bulk water flow measured in the present study.

The only previous study¹³ examining ovine amniotic fluid in response to absent swallowing has several important differences from the present study. First, esophageal ligation was performed at the time of surgery rather than after postoperative recovery. Second, absent fetal swallowing was not confirmed. Third, fetal

Table III. Amniotic fluid values before (day 1) and after (day 4) esophageal occlusion

	Day 1	Day 4
Osmolality (mOsm/kg)	275 ± 3	278 ± 5
Sodium (mEq/L)	129.1 ± 2.6	130.1 ± 3.4
Potassium (mEq/L)	4.9 ± 0.6	6.4 ± 0.6
Chloride (mEq/L)	105.6 ± 3.3	110.8 ± 2.5

urine production was not determined, and, finally, the amniotic fluid volume was measured only at delivery weeks later. The present study demonstrates increased amniotic fluid volume after 3 days of esophageal occlusion, a time period not previously examined. The single fetus in which the esophagus was occluded at surgery demonstrated increased amniotic fluid volume on study day 1. Although potential compensatory changes in amniotic fluid production were not determined by Wintour et al.,13 the finding of normal amniotic fluid volume at delivery suggests that during chronic esophageal ligation alternative routes account for amniotic fluid resorption. The present study suggests a mincr contribution of fluid resorption from alternative routes as the increase in amniotic fluid volume (948 ml) was less than the sum of extrapolated urine volume and estimated lung fluid production rates.5 Together, these studies suggest that inhibition of ovine fetal swallowing may result in short-term, perhaps transient, polyhydramnios similar to that noted in the rhesus monkey. 12

In spite of the increase in amniotic fluid volume, there was no change in amniotic fluid composition. During the 4 days (80 hours) of study, fetal urine contributed (extrapolated) a net of 287 Osm and 1117 ml to the amniotic fluid, yielding an average urine osmolality of 257 mOsm/kg. This urine contribution, together with isotonic lung fluid, would likely maintain normal amniotic fluid osmolality (275 mOsm/kg) during the study period. Alternatively, amniotic fluid osmolality may be maintained by exchange of fluid and electrolytes to the mother⁹ or fetus⁸ across the amniotic membranes.

In the absence of swallowing, the fetus must acquire fluid by alternative routes to maintain urine and lung fluid production. The likely sources for this fluid influx include intramembranous or transplacental flow. As discussed above, intramembranous flow did not compensate for the increased amniotic fluid volume. Gresham et al. similarly concluded that the fetus is able to acquire large amounts of water during an 18-day period of continuous urine drainage (9250 ml). As they noted oligohydramnios at delivery, intramembranous flow or fetal swallowing could not have provided the fetus sufficient water. Thus we hypothesize that increased placental flow may provide the fetus sufficient body water to continue urine and lung fluid production in the absence of swallowing. The driving forces for increased placental flow are poorly understood, however.26 Nevertheless, the ability of the fetus to adapt to the presence or absence of significant volumes of ingested fluid illustrates the dynamic nature and potential magnitude of transplacental flow capabilities.

In summary, fetal esophageal occlusion evoked urinary hyperosmolality and markedly increased amniotic fluid volume within 3 days. These results indicate an important role of fetal swallowing in amniotic fluid volume homeostasis.

We thank Linda Day for technical assistance.

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⁶⁶I think we've been stood up."

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg). Where protection begins

The physician should become familiar with the prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks), risk of estrogen dose, and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued when appropriate.

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Cigarette-smeking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhage stroke, myocardial infraction, visual disorders, hepatic tumors, galibladder disease, hypertension, tetal abnormalities. Practitioners should be familiar with the available information relating to

these and other risks.

1. **Inromboembolic disorders** and other vascular problems: Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE), British and U.S. studies showed increased risk for ratal venous thromboembolism (TE), British and U.S. studies showed increased risk for ratal venous thromboembolism (TE), British and U.S. studies showed increased risk for stoke. OC versil excess mortality from PE or stoke was 16.34, details/year/100,000 users and increased risk for thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stoke was 1.3-34, details/year/100,000 users and increased with age. *Cerebrovascular Disorders**. In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries incideate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPG (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takors is evident, but the numbers are too small to justily an estimation of the degree of risk." A 1981 analysis showed 4 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thromasis or embolism and TIA. Peripheral arterial diseases and MI were increased. Women over 30 had more arterial diseases, being greatest in sures who smoked. Family Planning Assoc, (PFA) found significant Mortality from nomenumatic heart disease was greater in ever-users. In the Walmut Creek Study, subarachnoid hemorrhage risk was ass

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year									
	Aged 30-39:	Users	Nonusers	Aged 40-44:	Users	Nonusers			
Ali smokers	•	10.2	2.6	-	62.0	15.9			
≥15 cigarettes daily		13,0	5.1		78.7	31.3			
<15 cigarettes daily		4.7	0.9		28.6	5.7			
Nonsmokers		1.8	1.2		10.7	7.4			
Smokers and nonsmokers		5.4	1.9		32.8	11.7			

Nonsmokers 1.8 1.2 10.7 7.4

Smokers and nonsmokers 5.4 1.9 32.8 11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from marker research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including cornary thrombasis, in excess of that predicted by sales. OCs with ≥ 100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor: influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose no between enthinyl estraidol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not bendone. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and pose of different progestogens are ignored. OCs may be divided into those with <100 mcg and those with ≥ 100 mcg estrogen. For all cases combined, larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhermorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses, but confidence from the control of the cont

Risk is: A-very high; B-high; C-moderate; D-low.	Age:	<30	30-39	≥40
Heavy smokers (≥ 15/day)		С	В	A
Light smokers (<15/day)		D	C	В
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		С	C,B	B,A

Nonsmokers, with predispositions

Be alert to earliest manifestations of TE disorders (eg. thrombophlebitis, PE, cerebovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately, A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in protonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. 2. Doutar lesions. Neuro-ocular lesions such as optic neurities or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. 3. Cerzionara: Long-term estropen in certain animals increases carcinomas and ronomalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase encidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endomethial carcinoma. In case-controlled studies, increased endomethial carcinoma in Cese-controlled studies, increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcinoma in 600 supported by increased endomethial carcino

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Atrial natriuretic factor responses to volume expansion in pregnant and nonpregnant sheep

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Isotonic volume expansion results in atrial natriuretic factor release by cardiac myocytes. Because pregnancy produces well-established alterations in fluid homeostasis and cardiovascular function, changes in atrial natriuretic factor responses may also occur. This study compares plasma atrial natriuretic factor responses to short-term volume expansion in pregnant and nonpregnant sheep. Seven pregnant and six nonpregnant ewes were chronically instrumented and subjected to a series of four experiments consisting of a control group (no infusion) and groups that received 10 ml/kg, 25 ml/kg, and 40 ml/kg isotonic saline infusion over a 30-minute period. The order of the experiments was random and separated by ≥48 hours. Plasma atrial natriuretic factor, osmolality, right atrial pressure, blood pressure, and urine flow were measured over a 150-minute observation period. After volume expansion, plasma atrial natriuretic factor levels rose significantly from 39 \pm 4 pg/ml (mean \pm SEM) to 49 \pm 7 pg/ml, 36 \pm 4 pg/ml to 62 \pm 19 pg/ml, and 39 ± 6 pg/ml to 67 ± 14 pg/ml in the nonpregnant group 10 ml/kg, 25 ml/kg, and 40 ml/kg experiments, respectively. In the pregnant groups, plasma atrial natriuretic factor levels rose from 50 ± 2 pg/ml to 75 \pm 20 pg/ml, 43 \pm 5 pg/ml to 57 \pm 5 pg/ml, and 46 \pm 4 pg/ml to 67 \pm 7 pg/ml, respectively. Differences in atrial natriuretic factor responses were not seen between pregnant and nonpregnant groups at any volume expansion level. As expected, atrial pressure and urine flow significantly increased after all volume expansion experiments. Pregnant and nonpregnant groups were similar with respect to atrial pressure and urine flow responses. Over various volume expansion levels significant associations were seen between atrial pressure, atrial natriuretic factor, and urine flow. These relationships were unaltered by pregnancy. In summary, atrial natriuretic factor responses to volume expansion do not appear to differ between pregnant and nonpregnant sheep. (AM J OBSTET GYNECOL 1991;165:1627-34.)

Key words: Plasma volume, urine flow, atrial pressure, vascular capacitance, cardiac compliance

Atrial natriuretic factor (ANF) is a polypeptide hormone released from cardiac atria in response to a variety of stimuli. Release is believed to be primarily a result of atrial stretch, although other mechanisms may be involved. 1.2 Isotonic volume expansion, for example, results in ANF secretion by increasing atrial pressure and volume. 3 ANF has potent effects on the cardio-vascular and renal systems, including reductions in cardiac output and blood pressure and increases in vascular compliance and urine flow. 4 Pregnancy also produces well-established changes in cardiovascular and renal function. 5 Cardiac output, blood volume, and glo-

merular filtration rate increase from 30% to 50% by late gestation in most mammals. Capacitance and compliance of the peripheral vasculature are also increased. Such physiologic responses are important to the development of the fetus.

Pregnancies failing to demonstrate blood volume expansion result in perinatal morbidity.6.7 Therefore given the gradual volume expansion present in normal pregnancy it is reasonable to question whether the regulation of ANF is altered. Information regarding the effect that pregnancy has on ANF release is limited. Although Cusson et al.8 reported increased plasma ANF levels with advancing gestation, most subsequent studies have shown little or no difference in pregnant and nonpregnant states. 9-11 Even less is known about the effect of pregnancy on ANF response to known stimuli. The ANF response to volume expansion has not been examined in pregnancy. Possible alterations in ANF release during pregnancy have potentially significant clinical relevance. Common diseases of pregnancy, including hypertensive disease, preeclampsia, cardiomyopathy, and tocolytic-associated pulmonary edema, may partially represent pathologically altered

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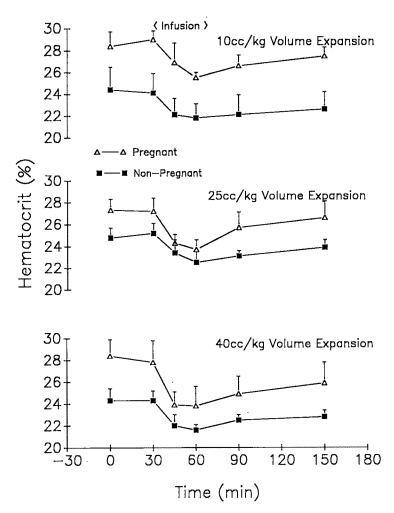


Fig. 1. Time course for hematocrit in 10 ml/kg, 25 ml/kg, and 40 ml/kg volume expansion experiments. Isotonic volume expansion began at 30 minutes and stopped at 60 minutes. After volume expansion, hematocrit fell at each volume expansion level (p = 0.05, 0.05, 0.01, respectively).

fluid homeostasis. Before any possible link between ANF and such conditions can be explored, the effects of normal pregnancy must be defined.

Our study is designed to compare plasma ANF levels in the pregnant and nonpregnant state and to examine the effect of pregnancy on ANF responses to volume expansion.

Material and methods

All procedures were performed with the approval of the Animal Care and Use Committee at Wake Forest University Medical Center. Seven pregnant ewes ranging from 110 to 139 days' gestation and six nonpregnant ewes were maintained in the facility several days before surgery. Each ewe was catheterized under general anesthesia. Polyvinyl catheters were placed in the fundus of the bladder for urine flow measurement and in the right atrium for pressure recording. Vascular catheters were inserted into a femoral vein for saline infusion and into a femoral artery for heart rate mon-

itoring blood pressure monitoring, and blood sampling. All catheters were exteriorized to the ewe's flank. Five days were allowed for surgical recovery.

Each ewe was subjected to a series of three volume expansion experiments and a control experiment. The order of these was randomly assigned and separated by at least 2 days. The experiments included a control (no infusion), 10 ml/kg, 25 ml/kg, and 40 ml/kg body weight isotonic saline infusion rapidly infused over 30 minutes. The time course consisted of an initial 30-minute observation period, a 30-minute infusion period, and a final 90-minute study period. All experiments were conducted with the ewes in standing position. Blood samples for ANF, hematocrit, and osmolality were drawn at 0, 30, 45, 60, 90, and 150 minutes. Urine volumes were measured at each of the above time segments and urine flow was calculated.

Plasma ANF was measured with radioimmunoassay techniques described elsewhere. ¹² Intraassay coefficient of variation was 8% and interassay 12%. Hematocrit

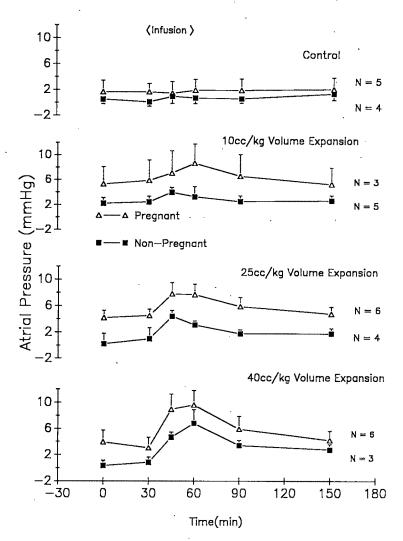


Fig. 2. Time course for right atrial pressure in control, 10 ml/kg, 25 ml/kg, and 40 ml/kg volume expansion experiments. Isotonic volume expansion began at 30 minutes and stopped at 60 minutes. Increases were noted after volume expansion (p = 0.02, 0.0001, 0.0001, respectively). No differences were noted between pregnant and nonpregnant groups at any volume expansion level.

Table I. Baseline values for control experiments

Nonpregnant (mean \pm SEM)	Pregnant (mean ± SEM)	p Valuc
36 ± 4	39 ± 4	NS
0.5 ± 0.5	1.7 ± 1.8	. NS
82 ± 4	102 ± 4	p = 0.01
25.8 ± 0.2	29.5 ± 1.9	NS
80 ± 4	85 ± 3	NS
1.5 ± 1.0	1.4 ± 0.4	NS
52.6 ± 6.8	64.2 ± 0.9	p < 0.05
	36 ± 4 0.5 ± 0.5 82 ± 4 25.8 ± 0.2 80 ± 4 1.5 ± 1.0	36 ± 4 39 ± 4 0.5 ± 0.5 1.7 ± 1.8 82 ± 4 102 ± 4 25.8 ± 0.2 29.5 ± 1.9 80 ± 4 85 ± 3 1.5 ± 1.0 1.4 ± 0.4

NS, Not significant.

was determined in duplicate with a microcapillary technique. Heart rate, arterial pressure, and atrial pressure were continuously monitored (recorder, Hewlett-Packard, Palo Alto, Calif.; IBM AT computer, IBM, Boca Raton, Fla.).

Because of the difficulty in maintaining catheters for extended periods of time, not all measurements were

available in each experiment. The number of measurements for each experiment is indicated in Figs. 1 through 5.

All data were expressed as mean ± SEM. Comparisons between nonpregnant and pregnant groups were made with a two-way analysis of variance with repeated measures for both volume expansion and time.13 Re1630 Bennett and Rose December 1991
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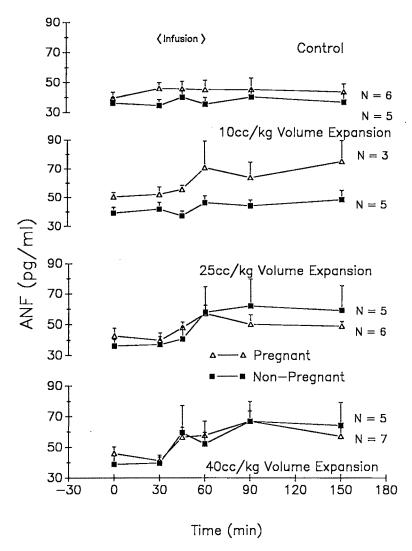


Fig. 3. Time course for plasma ANF in control, 10 ml/kg, 25 ml/kg, and 40 ml/kg volume expansion experiments. Isotonic volume expansion began at 30 minutes and stopped at 60 minutes. Increases were noted at each volume expansion level (p = 0.002, 0.002, and 0.003, respectively). No differences were noted between pregnant and nonpregnant groups at any volume expansion level.

lationships among atrial pressures, urine flows, and ANF responses were examined with regression analysis. Significance was set at $p \le 0.05$.

Results

Baseline values of all measured variables taken at the beginning of each control experiment are shown in Table I. Heart rate was higher in pregnant animals (p=0.01), but similar base values were seen for plasma ANF, right atrial pressure, hematocrit, mean arterial pressure, urine flow, and animal weight. After volume expansion, heart rate rose significantly in 25 ml/kg and 40 ml/kg volume expansion experiments. However, these changes were similar in pregnant and nonpregnant groups. An increase in mean arterial pressure was seen after volume expansion in 25 ml/kg and 40 ml/kg volume expansion experiments. Again, preg-

nant and nonpregnant groups failed to differ in mean arterial pressure response. As expected, hematocrit fell significantly in both pregnant and nonpregnant groups after volume expansion (Fig. 1). Although hematocrit values were slightly higher in the pregnant animals (p = 0.10), hematocrit changes were not different between groups for any volume expansion level.

Right atrial pressure rose significantly in all volume expansion experiments as compared with the control experiments (Fig. 2). Right atrial pressure had returned to baseline values by the conclusion of the experiment. At the beginning of all experiments right atrial pressure appeared higher in pregnant animals. This reached significance only in the 25 ml/kg volume expansion experiments. After volume expansion, however, changes in right atrial pressure were similar in pregnant and nonpregnant groups.

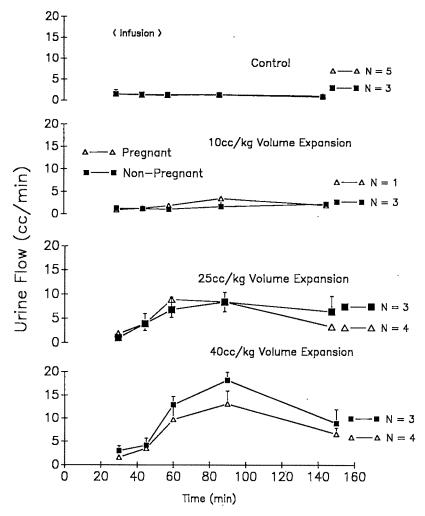


Fig. 4. Time course for urine flow in control, 10 ml/kg, 25 ml/kg, and 40 ml/kg volume expansion experiments. Isotonic volume expansion began at 30 minutes and stopped at 60 minutes. Increases in flow were seen at each volume expansion level (p = 0.002, 0.001, 0.001, respectively). Except for slightly increased response in nonpregnant group at 10 ml/kg volume expansion (p = 0.06), no differences were noted between pregnant and nonpregnant groups.

Baseline plasma ANF levels were not different between pregnant and nonpregnant groups (Fig. 3). After volume expansion, plasma ANF levels rose significantly from 39 ± 4 pg/ml (mean \pm SEM) to 49 ± 7 pg/ml, 36 ± 4 pg/ml to 62 ± 19 pg/ml, and 39 ± 6 pg/ml to 67 ± 14 pg/ml in the nonpregnant 10 ml/kg, 25 ml/kg, and 40 ml/kg experiments, respectively. In the pregnant groups, plasma ANF levels rose from 50 \pm 2 pg/ml to 75 \pm 20 pg/ml, 43 \pm 5 pg/ml to 57 \pm 5 pg/ml, and 46 \pm 4 pg/ml to 67 \pm 7 pg/ml, respectively. Although at 10 ml/kg volume expansion pregnant plasma ANF was slightly higher (p = 0.08) than the corresponding nonpregnant level, this trend was not seen at any other volume expansion level.

Prompt increases in urine flow were seen in all volume expansion experiments (Fig. 4). No significant differences were present between pregnant and nonpregnant groups for any volume expansion experiments.

Although all levels of volume expansion produced significant responses with regard to plasma ANF levels, atrial pressure, and urine flow, the 10 ml/kg and 25 ml/kg volume expansion experiments produced changes that were similar. At 40 ml/kg volume expansion, however, larger responses were seen in ANF (p = 0.05), atrial pressure (p = 0.01), and urine flow (p = 0.02).

When examining the effects of different variables on ANF response with multiple regression analysis, changes in right atrial pressure were most predictive of ANF response. Body weight, gestational age, heart rate, and pregnancy status all failed to appreciably relate to plasma ANF changes.

To analyze the relationship between plasma ANF, atrial pressure, and urine flow during volume expansion, maximal changes in these variables were compared. Fig. 5 shows the relationship between atrial pres-

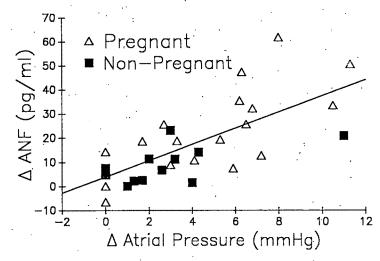


Fig. 5. Changes in right atrial pressure versus plasma ANF changes (r = 0.74, p = 0.0001). Values represent maximal changes in given variable during each experiment. All experiments are included for which measurements were available.

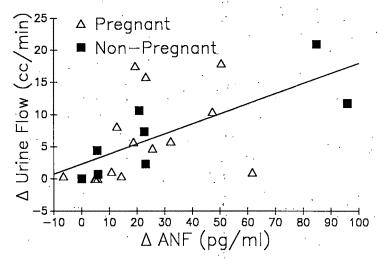


Fig. 6. Plasma ANF changes versus changes in urine flow (r = 0.62, p = 0.001). Values represent maximal changes in given variable during each experiment.

sure change and changes in ANF. The association appears to be linear and is similar in pregnant and nonpregnant experiments (r=0.74, p=0.0001). The regression line predicts an increase of 3.5 pg/ml in plasma ANF for every 1 mm Hg increase in right atrial pressure. The relationship between ANF response and urine flow changes is depicted in Fig. 6. Again, a linear association exists that is similar in pregnant and nonpregnant experiments (r=0.62, p=0.001). Besides ANF responses, right atrial pressure changes also predicted urine flow response as seen in Fig. 7 (r=0.72, p=0.001). This relationship was similar in pregnant and nonpregnant experiments.

Comment

In the current study pregnancy did not have a significant effect on basal plasma ANF levels or basal atrial pressures. Some investigators have postulated that ANF

levels should rise as a result of the large increase in plasma volume associated with pregnancy. This idea is consistent with the concept of pregnancy as an "overfill" state. Under such conditions ANF would rise as a result of increases in atrial pressure.18 However, our results agree with those of several studies mentioned previously that indicate little or no change in plasma ANF levels in pregnancy.9-11 These findings support pregnancy as an "underfill" state in which effective blood volume is not expanded. However, our understanding of the mechanism of ANF release and its metabolism is incomplete, and other explanations exist. Although atrial stretch is considered the chief stimulus for ANF release after volume expansion,3 the effects of pregnancy (specifically the hormonal changes accompanying pregnancy) on this mechanism remain unstudied. It can be speculated that homeostatic set-points undergo remodeling in the pregnant state. Also, ANF

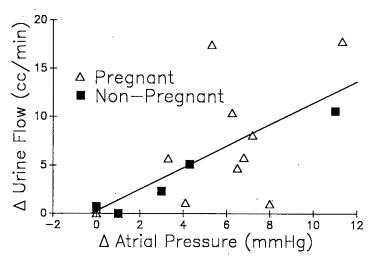


Fig. 7. Changes in right atrial pressure versus urine flow changes (r = 0.72, p = 0.001). Values represent maximal changes in given variable during each experiment.

release may increase during pregnancy but would not be reflected in increased plasma levels if clearance also increased. This is not without precedent, because the fetus demonstrates both increased release and clearance of this peptide as compared with the adult.14

Isotonic volume expansion produced significant elevations in plasma ANF at each volume expansion level in both the pregnant and nonpregnant groups. Thus these experiments confirm that pregnant animals exhibit an ANF response to volume expansion. In our experimental design volume expansion was normalized between pregnant and nonpregnant groups by adjusting the infused volume by body weight in keeping with the observations of Metcalfe and Parer¹⁵ that changes in blood volume are linearly correlated with body weight in sheep.15 Adjustment of each volume expansion level (10 ml/kg, 25 ml/kg, and 40 ml/kg body weight) resulted in very similar atrial pressure changes and dilutional changes in hematocrit in the pregnant and nonpregnant groups. Even though the pregnant animals received a larger absolute volume infusion, atrial pressures did not differ from nonpregnant animals and, most important, plasma ANF responses were not different. Because vascular capacitance is increased during pregnancy, effective blood volume may be reduced; it is possible that increased fluid loads might not be reflected by increased atrial pressure. Hatjis et al.12 reported no change in ANF levels in pregnant human subjects 20 minutes after acute volume expansion (25 ml/kg) in preparation for epidural anesthesia. The authors reasoned the lack of ANF response was caused by the enlarged (i.e., underfilled) vascular compartment.

Also, changes in cardiac compliance during pregnancy may alter the relationship between atrial pressure and atrial stretch. In these experiments, however, right atrial pressure changes were similar in pregnant and nonpregnant experiments, as were plasma ANF levels. In fact, the relationship between atrial pressure and ANF response was linearly correlated without respect to pregnancy status. This information suggests that in spite of all the changes in various hormones and volume status during pregnancy, the underlying mechanisms for ANF response to volume expansion remain intact and minimally altered.

After volume expansion, increases in urine flow may be partly attributable to plasma ANF increases, but many studies indicate such ANF effects on renal responses are minimal, compared with other hormonal and neural mechanisms. 16, 17 In this study a relationship was noted between ANF response and urine flow in both pregnant and nonpregnant groups. Although these experiments do not clarify whether this relationship is causal, they do suggest that the degree and type of relationship is unaltered by pregnancy.

Increases in volume expansion from 10 ml/kg to 25 ml/kg failed to significantly increase atrial pressure. It is not surprising that ANF also failed to show a larger response. When atrial pressure did increase significantly at 40 ml/kg volume expansion, ANF similarly changed. This finding supports atrial pressure as the primary stimulus for ANF release during volume expansion. Pregnancy demonstrated little impact on this interaction.

In summary, plasma ANF increased in adult sheep after isotonic volume expansion. Pregnancy appeared to have no effect on ANF response, nor did it affect relationships between atrial pressure and ANF response and between ANF response and urine flow.

We thank Drs. Frank Greiss, Jr., and Paul J. Meis for their support.

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Fetal and maternal plasma atrial natriuretic factor responses to angiotensin II infusion

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Plasma atrial natriuretic factor and angiotensin II have opposing actions in the regulation of body fluid homeostasis and systemic blood pressure. Angiotensin II infusions stimulate atrial natriuretic factor release in some, but not all, studies in adult mammals. To examine the response during the perinatal period, graded intravenous angiotensin II infusions were administered to four chronically instrumented pregnant ewes and fetuses (132 ± 1 days of gestation). Fetuses received successive 20-minute intravenous angiotensin II infusions at 25, 50, and 100 ng/kg/min. After a 90-minute recovery, maternal ewes received successive 20-minute angiotensin II infusions (5, 10, and 25 ng/kg/min). During the fetal infusions, mean (\pm SEM) fetal arterial blood pressure (47 \pm 2 to 61 \pm 2 mm Hg, ρ < 0.05) and heart rate (155 \pm 16 to 196 \pm 36 beats/min, p < 0.05) increased, although there was no change in maternal measured parameters. In response to the maternal infusion, maternal mean arterial blood pressure increased (92 \pm 7 to 110 \pm 8 mm Hg, p < 0.05) and maternal heart rate decreased (136 \pm 4 to 125 \pm 2 beats/min, p < 0.05) without change in fetal parameters. Fetal plasma atrial natriuretic factor levels $(210 \pm 27 \text{ to } 664 \pm 250 \text{ pg/ml}, p < 0.05)$ significantly increased during the fetal angiotensin II infusions in spite of no change in maternal plasma atrial natriuretic factor (85 ± 21 to 124 ± 22 pg/ml) during the maternal angiotensin II infusions. These findings indicate that similar increases in systemic blood pressure in response to angiotensin II infusions stimulate increased fetal, but not maternal, plasma atrial natriuretic factor. (AM J OBSTET GYNECOL 1991;165:1635-41.)

Key words: Angiotensin, atrial natriuretic factor, ovine fetus

The renin-angiotensin-aldosterone system and atrial natriuretic factor (ANF) interact in the regulation of body fluid homeostasis. Increased plasma ANF generally acts to decrease body water by urinary diuresis and natriuresis and to stimulate plasma-to-interstitial fluid flow.1.2 Conversely, aldosterone promotes renal sodium retention and extracellular volume expansion. During pregnancy these systems may contribute to maternal and fetal fluid homeostasis. Stimulation of the maternal renin-angiotensin-aldosterone system may be essential for appropriate maternal plasma volume expansion during pregnancy.3 In the puerperium increased maternal plasma ANF4 and suppression of renin³ may promote maternal diuresis and natriuresis, returning blood volume toward nonpregnant levels. Similarly, fetal renin-angiotensin and ANF may modulate in utero renal function and cardiac output distribution,⁵⁻⁸ whereas increased neonatal plasma ANF may contribute to the changes in newborn body water composition.⁹

The secretion of ANF and renin-angiotensin may also be interdependent. ANF has been demonstrated to inhibit renin secretion and angiotensin-mediated aldosterone release in vivo and in vitro. ¹⁰ Angiotensin II infusions stimulate ANF release in some, ¹¹ but not all, ¹² studies in adult mammals. However, the effect of angiotensin II infusion on plasma ANF has not been studied during pregnancy. In view of the potential importance of this interaction to fetal and maternal fluid homeostasis, we examined the ovine fetal and maternal plasma ANF responses to graded angiotensin II infusions.

Material and methods

Animals and surgery. Four Western cross-bred ewes and fetuses were studied. Guidelines for the care and use of animals approved by Harbor-University of California, Los Angeles Medical Center were followed, and the experimental procedures and protocol were approved by the Harbor-University of California, Los Angeles Animal Care Committee. Animals were maintained under controlled photoperiods of 12 hours light/12 hours dark and housed in individual steel study cages before and after operation. Both food (al-

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falfa pellets) and water were freely available, except for the withholding of food during the 24-hour period before surgery. Anesthesia was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg) plus atropine sulfate (30 µg/kg) and subsequently maintained by a continuous intravenous infusion of ketamine (15 mg/kg/hr). Polyethylene catheters (inner diameter 1.0 mm, outer diameter 1.8 mm) were placed in the fetal dorsal hind limb artery and vein and threaded to the abdominal aorta and inferior vena cava, respectively. A plastic catheter (Corometrics Medical System, Wallingford, Conn.) was sutured to the distal tip of the fetal hind limb for measurement of intrauterine pressure, after which the uterus and the maternal abdominal wall were closed in layers. The maternal femoral artery and vein were catheterized and all catheters were exteriorized to the maternal flank and placed in a cloth pouch sewn to the ewe's flank. As described previously,13 all animals were provided a minimum of 5 days' postoperative recovery, during which time antibiotics were administered and catheters flushed.

Experimental protocol

Fetal infusion. All experiments were performed on unanesthetized ewes standing in the same individual cages in which they were maintained. The mean gestational age at the initiation of studies was 132 ± 1 day of gestation. On the day of study, fetal and maternal heart rate and blood pressure and amniotic fluid pressure were monitored during a 30-minute control period. Basal samples of maternal and fetal arterial blood (2.5 ml) were drawn at -30 minutes for measurement of hematocrit and plasma ANF level. An additional 0.5 ml sample of arterial blood was collected for immediate blood gas analysis. At time 0, intravenous infusions of angiotensin II in 0.15 mol/L saline solution (2 µg/ml) were administered to the fetus at 25, 50, and 100 ng/kg/min (maximum rate 0.05 ml/kg/min) for 20 minutes each. Before the infusions, the fetal catheters were filled with a volume of the angiotensin II solution equal to the catheter's dead space (1.0 ml). After the infusions, fetuses were monitored for an additional 30minute recovery period. Fetal body weight was estimated from the data of Robillard et al.14

FBW (kg) =
$$[0.096 \times \text{gestational age}]$$

(days)] - 9.2228

where FBW is fetal body weight. The angiotensin II infusate was prepared on the day of infusion by dissolving the preweighed 5-valine angiotensin II (Bachem, Torrance, Calif.) in normal saline solution. Fetal blood samples for hematocrit, plasma ANF, pH, and blood gases were obtained at 20, 40, 60, and 90 minutes after initiation of the infusion. Maternal blood samples were repeated at the conclusion of the final infusion (60 minutes).

Maternal infusion. After the fetal recovery period and an additional 1-hour period, intravenous infusions of angiotensin II (5, 10, and 25 ng/kg/min) were administered to the maternal ewes by an identical protocol. The reduced maternal angiotensin II doses were selected to achieve equivalent dose-dependent increases in fetal and adult plasma angiotensin II concentrations. Throughout both studies, all fetal blood samples were immediately replaced with equivalent volumes of heparinized maternal blood drawn before the study, and all maternal samples were immediately replaced with equivalent volumes of saline solution.

Analytic methods. Maternal and fetal heart rate and blood pressures and amniotic fluid pressures were monitored with a Beckman R-612 physiologic recorder (Beckman, Palo Alto, Calif.) and Statham P-23 DB pressure transducers (Gould, Inc., Cleveland). Fetal blood pressure was corrected for amniotic fluid pressure. Blood samples were transferred immediately to chilled test tubes containing aprotinin (250 kallikrein units per milliliter of blood), trypsin inhibitor (40 µU/ml), and 15% ethylenediaminetetraacetic acid (10 µl/ml) for determination of plasma ANF. All tubes were mixed and centrifuged immediately at 4° C, and the plasma was separated and frozen for later assay. Hematocrit determinations were performed in duplicate on each blood sample. Blood pH, Po2, and Pco2 values were measured at 39° C with a model BM 33 MK2-PHM 72 MKS acid-base analyzer system (Radiometer Co., Copenhagen).

Plasma ANF concentrations were measured by radioimmunoassay as previously described.¹⁵ The ANF radioimmunoassay is sensitive to 10 pg/ml, with intraassay and interassay coefficients of variation of 5% and 11%, respectively.

Calculations and statistics. All values are expressed as the mean \pm SEM. Plasma ANF levels were log transformed after determination of nonnormality by the Wilk-Shapiro test. Differences over time were assessed by analysis of variance with trends in the data considered statistically significant when the F test yielded a p < 0.05. Differences between time periods were determined with Dunnett's test at a p < 0.05 level of significance. For the analysis of angiotensin II dose-response effects, linear regression was performed for maternal and fetal blood pressures (systolic, diastolic, and mean) versus angiotensin II infusion doses.

Results

Fetal infusions. In response to the intravenous angiotensin II infusions, fetal systolic, diastolic, and mean blood pressures significantly increased (Fig. 1, Table I) and returned to basal values during the recovery period. Regression analysis revealed significant correlations of fetal mean systolic and diastolic blood pressures with angiotensin II infusion doses; r values ranged from

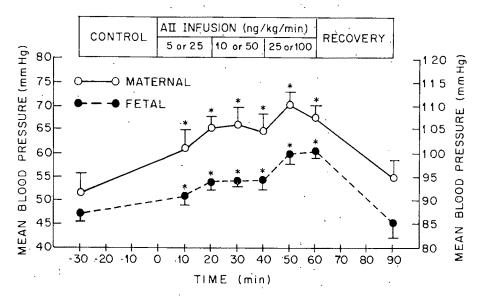


Fig. 1. Maternal (solid line, right vertical axis) and fetal (dashed line, left vertical axis) mean arterial pressure before, during, and after graded intravenous angiotensin II infusions.

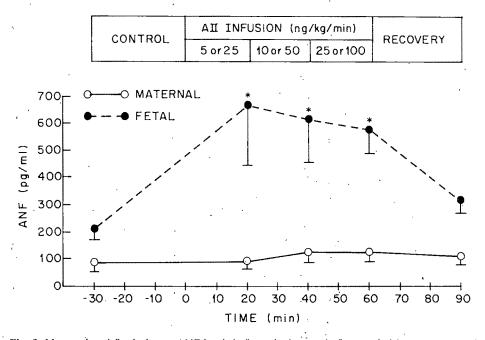


Fig. 2. Maternal and fetal plasma ANF levels before, during, and after graded intravenous angiotensin II infusions.

0.69 to 0.80 (Table II). Basal fetal plasma ANF (210 ± 27 pg/ml) significantly increased more than threefold in response to the 25 ng/kg/min angiotensin II infusion (Fig. 2). In spite of continued increases in fetal blood pressure, no further change in fetal plasma ANF occurred. Plasma ANF returned toward basal values at the conclusion of the recovery period. Fetal heart rate increased significantly in response to the 50 and 100 ng/kg/min infusion rates (Table I). Fetal hematocrit, pH, PO2, and PCO2 did not change during the study.

There was no change from basal levels of maternal hematocrit (30.5% \pm 2.6%), pH (7.46 \pm 0.05), PO₂

 $(99 \pm 3 \text{ mm Hg})$, Pco_2 $(35 \pm 4 \text{ mm Hg})$, mean blood pressure (96 \pm 12 mm Hg), or heart rate (148 \pm 4 beats/min) during the fetal angiotensin II infusion.

Maternal infusions. In response to the angiotensin II infusions to the ewe, maternal systolic, diastolic, and mean blood pressures significantly increased (Fig. 1, Table III). Increases in maternal mean and diastolic, but not systolic, blood pressures were significantly correlated with the angiotensin II infusion doses. However, r values for mean and diastolic blood pressures were only 0.44 and 0.50, respectively (Table II). Maternal heart rate decreased significantly in response to 1638 Ross et al.

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Table I. Fetal arterial values before	during, and after fetal	intravenous angiotensin II infusions
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			Infusate					
	Before infusate	25 ng/	kg/min	50 ng/	kg/min	100 ng	/kg/min	After infusate
Time (min) Blood pressure	-30	10	20	30	40	50	60	90
(mm Hg) Systolic Diastolic	56 ± 1 39 ± 2	64 ± 2* 43 ± 2	64 ± 3* 45 ± 2	66 ± 2* 46 ± 2*	65 ± 2* 48 ± 3*	73 ± 1* 51 ± 2*	72 ± 1* 52 ± 2*	55 ± 5 39 ± 3
Heart rate (beats/min) Hematocrit	155 ± 16 37.0 ± 1.4	152 ± 22 37.3 ± 1.4	151 ± 25 37.8 ± 1.4	179 ± 32 38.0 ± 1.2	$196 \pm 36*$ 37.8 ± 1.3	181 ± 23 37.8 ± 1.5	$192 \pm 19*$ 37.8 ± 1.3	177 ± 12 37.5 ± 1.3
(%) pH	7.36 ± 0.02	7.41 ± 0.03	7.42 ± 0.03	7.43 ± 0.04	7.43 ± 0.03	7.42 ± 0.03	7.42 ± 0.03	7.42 ± 0.04
PO_2 (mm Hg) PCO_2 (mm Hg)	18 ± 2 46 ± 1	16 ± 2 43 ± 1	16 ± 3 43 ± 1	17 ± 3 42 ± 1	16 ± 3 42 ± 1	16 ± 3 42 ± 1	15 ± 2 42 ± 1	16 ± 2 43 ± 1

^{*}p < 0.05, versus - 30-minute value.

Table II. Linear regression results of fetal and maternal blood pressure (mean, systolic, diastolic) versus angiotensin II infusion doses

	Regression equation	p Value	r Value	
Fetal				
Mean	0.121x + 48.28	< 0.0001	0.80	
Systolic	0.144x + 58.32	< 0.0001	0.80	
Diastolic	0.116x + 40.31	< 0.0001	0.69	
Maternal				
Mean	0.483x + 98.19	0.019	0.44	
Systolic	0.212x + 118.04	0.387	0.17	
Diastolic	0.551x + 83.85	0.007	0.50	

the highest-dose angiotensin II infusion (Table III). Basal maternal plasma ANF levels ($85 \pm 21 \text{ pg/ml}$), hematocrit, pH, PO₂, and PCO₂ did not change during the study (Table III).

There was no change from basal levels of fetal hematocrit (37.1% \pm 1.4%), pH (7.39 \pm 0.02), PO₂ (19 \pm 2 mm Hg), PCO₂ (44 \pm 2 mm Hg), mean blood pressure (49 \pm 2 mm Hg), or heart rate (166 \pm 6 beats/min) during the maternal angiotensin II infusion.

Comment

Infusion of angiotensin II to the ovine fetus and ewe resulted in similar (10 to 20 mm Hg) increases in maternal and fetal mean blood pressures. The higher fetal angiotensin II infusion rates we used were due to the increased plasma clearance rate in late gestation ovine fetuses, as compared with adult ewes. Using similar fetal (26, 48, 97 ng/kg/min) and adult (5, 10, 20 ng/kg/min) angiotensin II infusion rates, Robillard et al. Achieved nearly equivalent dose-dependent increases in fetal and adult plasma angiotensin II concentrations from approximately 50 to 350 pg/ml; peak levels were similar to those measured in response to hemorrhage or hypotension. The 20-minute infusion

period was based on previous studies demonstrating steady-state plasma angiotensin II levels and stable measurements of mean arterial blood pressure at 10, 15, and 20 minutes of intravenous infusion.¹⁵

Fetal mean, systolic, and diastolic arterial blood pressures displayed significant dose-dependent increases in response to angiotensin II infusions (Table II). Notably, absolute increases in fetal arterial blood pressure were less than those demonstrated in recent fetal angiotensin II infusion dose-response studies. ^{16, 17} However, Clark et al. ¹⁶ used dosages of 100 to 3000 ng/kg/min, whereas Yoshimura et al. ¹⁷ used dosages ranging from 3 log units, with fetal dosages approaching 1500 ng/kg/min. Our results are consistent with these studies in demonstrating a statistically significant, although smaller, blood pressure dose response within a physiologic range of fetal angiotensin II infusions (25 to 100 ng/kg/min).

As a result of the nearly equivalent absolute increases in fetal and maternal blood pressures, there was a smaller relative increase in maternal blood pressure. Although angiotensin II infusions did induce dose-dependent increases in maternal mean and diastolic blood pressures, the correlations were not as great as with fetal blood pressures. Perales et al.¹⁸ previously demonstrated dose-related increases in mean arterial pres-

Table III. Maternal arterial values before, during, and after maternal intravenous angiotensin II infusions

		Infusate						
	Before infusate	5 ng/i	kg/min	10 ng/	kg/min	25 ng/	kg/min	After infusate
Time (min) Blood pressure (mm Hg)	-30	10	20	30	40'	50	60	90
Systolic Diastolic	111 ± 9 76 ± 6	121 ± 7* 86 ± 4*	124 ± 5* 91 ± 3*	124 ± 7* 94 + 4*	120 ± 6* 93 ± 5*	122 ± 4*	122 ± 4*	111 ± 6
Heart rate (beats/min)	136 ± 4	134 ± 4	126 ± 2	128 ± 3	135 ± 6	$97 \pm 4*$ $125 \pm 4*$	95 ± 4* 125 ± 2*	81 ± 3 136 ± 5
Hematocrit	30.5 ± 2.9	29.3 ± 2.4	30.3 ± 2.5	29.0 ± 2.4	29.3 ± 2.9	29.3 ± 2.5	28.5 ± 2.5	29.0 ± 2.4
pН	7.44 ± 0.01	7.43 ± 0.01	7.45 ± 0.02	7.46 ± 0.01	7.47 ± 0.01	7.47 ± 0.01	7.46 ± 0.01	7.46 ± 0.0
PO ₂ (mm Hg)	98 ± 7	99 ± 6	103 ± 2	104 ± 2	106 ± 6	100 ± 4	100 ± 1	102 ± 4
PCO ₂ (mm Hg)	31 ± 2	31 ± 3	31 ± 3	32 ± 3	31 ± 6	30 ± 5	31 ± 5	31 ± 4

^{*}p < 0.05, versus -30-minute value.

sure in pregnant ewes, although the dosages administered (1.15, 2.29, and 11.5 µg/min) were up to tenfold greater than the dosages in our study or that of Robillard et al.8 Notably the lowest dose used by Perales et al. (1.15 µg/min) achieved a similar increase in maternal mean arterial blood pressure as the maximum dose in our study. Bruce et al.19 similarly demonstrated dosedependent increases in ovine maternal blood pressure using a dosage range of 2 to 200 ng/kg/min. However, a review of their results¹⁷ indicates minimal increases in maternal blood pressure within the dosage range of 2 to 25 ng/kg/min. Importantly, Naden et al. 15 reported a markedly reduced angiotensin II pressor response during ovine pregnancy. Although it is possible that a tachyphylaxis to the successive infusions accounted for the reduced maternal blood pressure response, downregulation of angiotensin II receptor number likely would require longer than 20-minute infusion periods.20

Consistent with previous reports, 7.8, 18 the current experiments were notable for significant increases in fetal heart rate, with a small, although significant, decrease in maternal heart rate. Although the mechanism of the positive fetal chronotropic response is not clear, it may result from direct cardiac effects of angiotensin II or alterations in sympathetic and parasympathetic tone.7 The negative maternal chronotropic response is likely baroreceptor mediated. Neither the current study nor previous reports8, 18 detected a change in fetal or maternal blood gases, pH, or hematocrit during the angiotensin II infusions.

The angiotensin II infusions resulted in a significant increase in fetal, but not maternal, plasma ANF. ANF is present and may be released from both left and right adult atria21 in response to atrial stretch or increased atrial pressure.22 In response to angiotensin II-induced arterial constriction, increased cardiac afterload, left ventricular end-diastolic pressure, and perhaps left atrial pressure may be responsible for ANF release in dogs23 and rats.11 Conversely, norepinephrine, but not angiotensin II, increased left atrial echocardiographic area and plasma ANF in human volunteers, in spite of similar elevations in mean arterial pressure.¹² In the absence of atrial and venous pressure measurements in our study, the regulation of ovine ANF release by atrial pressure cannot be determined. Nevertheless, the results of this study suggest that the behavior of the adult sheep is similar to that of the human in failing to exhibit an ANF response to angiotensin II. Alternatively, the maternal ANF response may be a secondary effect of the reduced maternal angiotensin II pressor effect; the blood pressure threshold for maternal ANF release may be greater than that achieved in the present study.

Fetal plasma ANF levels increased threefold at the lowest angiotensin II infusion rate and remained elevated throughout the higher-dose infusions. The increase in fetal, but not maternal, ANF levels suggests differences in either the distribution and content of cardiac ANF or the cardiovascular response to angiotensin II. The ontogeny of ANF in the developing heart has been examined in both human and rat fetuses. In fetal and neonatal rat atria, relative ANF content (concentration per milligram of protein) increases with time.24 Fetal rat ventricular ANF content is high, although it rapidly decreases to minimal values in the adult.24, 25 In the developing human heart, right and left ventricular ANF contents similarly decrease with advancing gestation. 19, 26 Right atrial ANF content has been demonstrated as both greater19 and less26 than left atrial content during fetal life. In response to the fetal angiotensin II infusion, increased cardiac afterload and systemic blood pressure likely resulted in increased ventricular end-diastolic pressure. Although afterloadinduced ventricular ANF release has not been demonstrated, it is possible that fetal ventricular ANF contributed to the increased plasma ANF concentration in response to the angiotensin II infusion. In addition, reduced ANF clearance, in association with angiotensin II—mediated decreases in renal and placental blood flow,⁷ may also have contributed to the increase in plasma ANF.

The fetal angiotensin II infusion was administered before the maternal infusion in all cases to avoid a potential efect of maternal angiotensin II on fetal oxygenation and heart rate. However, decreased uterine blood flow and fetal effects of maternal angiotensin II infusions have been demonstrated only in response to pharmacologic dosages (110 to 200 ng/kg/min¹⁷ or 11.5 µg/min¹⁶). There was no evidence of altered fetal oxygenation in response to fetal or maternal angiotensin II infusions; therefore hypoxia does not appear to have contributed to fetal ANF release.²⁷

The stimulation of plasma ANF by angiotensin II infusion in the fetus but not the pregnant ewe has several important implications. First, relatively minor degrees of fetal hemorrhage result in a marked increase in plasma angiotensin II levels,28 which contribute importantly to fetal cardiovascular homeostasis.29 Increased fetal plasma angiotensin II results in significant changes in fetal cardiac output and blood flow distribution. As ANF has significant fetal cardiovascular effects, the angiotensin II-induced alterations may be mediated in part by ANF release. Second, the lack of a maternal ANF response to angiotensin II infusion may be a reflection of the reduced pressor sensitivity during pregnancy. 15 Mechanisms postulated for the reduced pressor responsiveness of pregnancy have included effects of increased prostacyclin, estrogen, and progesterone and down-regulation of angiotensin II receptors.30 Whether a greater increase in maternal blood pressure would have stimulated ANF is uncertain. However, because ANF may stimulate natriuresis, diuresis, and loss of intravascular volume, a maternal resistance to angiotensin II-stimulated ANF release would be of protective value in the hemorrhage-prone state of pregnancy.

In conclusion, the current study demonstrates significant increases in fetal, but not maternal, plasma ANF levels in response to similar, absolute increases in systemic blood pressure during angiotensin II infusion.

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Angiotensin II vascular smooth-muscle receptors are not down-regulated in near-term pregnant sheep

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Normal human and ovine pregnancies are associated with elevated plasma angiotensin II levels and refractoriness to the vasoconstrictor effects of infused angiotensin II, which is greater in the ovine uteroplacental vascular bed than in the systemic vasculature. It remains unclear whether this refractoriness reflects alterations in angiotensin II vascular smooth-muscle receptor density or affinity. We examined the angiotensin II vascular smooth-muscle receptor in nonpregnant (n=12) and near-term pregnant (130 ± 3 days [mean \pm SD], n=10) sheep, comparing binding characteristics on plasma membranes prepared from the medial layer of aorta, mesenteric artery, and uterine artery. Plasma angiotensin II levels were increased threefold to fourfold (p<0.001) in pregnant ewes. A single class of high-affinity angiotensin II vascular smooth-muscle receptor was identified in each type of artery. Receptor density was similar in nonpregnant and pregnant mesenteric artery (92 ± 21 vs 103 ± 40 fmol/mg protein, respectively), aorta (186 ± 29 vs 220 ± 46 fmol/mg protein), and uterine artery (59 ± 20 vs 77 ± 20 fmol/mg protein) tissue. Receptor affinity also was unchanged during pregnancy. Because changes in the density and affinity of the angiotensin II vascular smooth-muscle receptor were not observed in near-term pregnant ewes, the attenuated vasoconstrictor responses seen during pregnancy do not reflect receptor down-regulation or decreased affinity. (AM J OBSTET GYNECOL 1991;165:1641-8.)

Key words: Angiotensin II receptor—binding capacity, vascular receptors, sheep, uterine artery, pregnancy

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Normal pregnancy is characterized by numerous cardiovascular and hormonal alterations such as increases in cardiac output, decreases in systemic vascular resistance, development of the low-resistance uteroplacental vascular bed, and activation of the renin-angiotensin system, which is reflected by increased circulating levels of plasma renin activity and angiotensin II. Of particular interest has been the observation that normal pregnancy is also associated with the development of relative

refractoriness to the systemic pressor effects of infused angiotensin II.² This aspect of cardiovascular adaptation has been of substantial importance because this refractoriness to angiotensin II is lost early in the midtrimester in women destined to develop pregnancy-induced hypertension well before they demonstrate an elevation in blood pressure.² The mechanism(s) responsible for this refractoriness to infused angiotensin II during normal pregnancy and for its absence in women who have pregnancy-induced hypertension remains unclear.

Although pregnant sheep do not develop pregnancyinduced hypertension, they have been observed to develop many of the cardiovascular and hormonal changes reported in normal human pregnancy.3 The pregnant ewe,4.5 like women2 and several other species,6,7 develops refractoriness to infused angiotensin II in the face of increased plasma levels of this peptide and plasma renin activity.8, 9 Moreover, the dose-response curves and the effective pressor dose of angiotensin II, that is, the dose necessary to elicit a 20 mm Hg rise in blood pressure in the ewe, are strikingly similar to those reported in women.2,4 This model, however, is unique in that uteroplacental blood flow can be continuously monitored, and we have shown that this vascular bed is even more refractory to the vasoconstrictor effects of angiotensin II than the systemic vascular bed.10, 11 Using this model to study adaptive mechanisms in normal pregnancy, we have demonstrated that the attenuated systemic pressor response to angiotensin II does not reflect increases in the baroreceptor reflex12 or increased clearance of the peptide.9 We⁸ also have confirmed observations in normal pregnant women¹⁵ that acute decreases in plasma renin activity do not alter pressor responses to infused angiotensin II; these observations suggest that down-regulation of the angiotensin II vascular smooth-muscle receptor may not explain this phenomenon. This conclusion, however, is in conflict with that of Siddiqi et al.,14 who demonstrated an increase in vascular responsiveness to infused angiotensin II in pregnant ewes after 24 hours' treatment with enalapril, an angiotensinconverting enzyme inhibitor. In the few reports in which the angiotensin II receptor has been studied, alterations in nonvascular smooth-muscle angiotensin II receptors, but not the vascular smooth-muscle receptor, has been examined.7, 15, 16 Thus we sought to determine whether the attenuated responses to angiotensin II observed in the uteroplacental and systemic vascular beds of the pregnant ewe reflect down-regulation of the angiotensin II vascular smooth-muscle receptor and, if so, whether this is different in the uterine and systemic vascular beds.

Material and methods

Pregnant (n = 10) and nonpregnant (n = 12) ewes

versity of Texas Bastrop breeding facility and maintained on standard animal chow (Purina Commercial Creep II, G, Purina Mills, St. Louis) and given free access to water until killed (130 \pm 3 days, term 144 \pm 3 days, mean \pm SD). Animals were placed into two study groups: In the first group we examined the systemic vascular smooth-muscle receptor in the mesenteric artery and aorta of pregnant (n = 5) and nonpregnant (n = 6) ewes. In the second group we examined the uterine and mesenteric arteries in similar numbers of pregnant and nonpregnant animals. This separation into two groups was necessitated by the need to simultaneously study two arteries in each animal. After jugular venous blood was obtained for measurement of plasma angiotensin II animals were killed by the rapid intravenous injection of pentobarbital sodium (50 mg/kg). Immediately thereafter the mesenteric arteries extending from the first- to the third-generation arcades and the entire abdominal aorta were removed. For studies of the uterine arteries, vessels extending from the first to the fourth generations were obtained. Arteries used in these studies were dissected and removed from all animals by the same investigators (H.R.M. and R.R.M.) to ensure that the areas sampled were the same. Arteries were removed with minimal trauma, immediately placed in ice-cold 8 mmol/L phosphate-buffered saline solution (pH 7.4), and rinsed free of residual blood. Fat, connective tissue, and adventitia were dissected from the arteries and the endothelium removed with a cotton-tipped applicator. The remaining medial layer provided the source of smooth muscle examined. These studies were approved by the Institutional Review Board for Animal Research.

Receptor radioligand binding studies. After removal of the endothelium, the medial smooth muscle (1.0 to 1.5 gm wet weight) was transferred into 20 ml of ice-cold 0.25 mol/L sucrose with 10 mmol/L Tris buffer, pH 7.4, and minced. Plasma membranes were prepared at 4° C by modifications of the methods of Baker et al.17 With a probe (Polytron PT-20, Brinkman Instruments, Westbury, N.Y.), tissue was homogenized three times for 8 seconds; the probe was allowed to cool between homogenizations. The protease inhibitors phenylmethylsulfonyl fluoride (0.5 mmol/L), leupeptin (5 μ g/ml), and aprotinin (5 μ g/ml) were added to the homogenate. The homogenate was centrifuged at 4° C at 10,000g for 20 minutes, and the supernatant was decanted, filtered through two layers of gauze, and centrifuged at 4° C at 45,000g for 30 minutes. The resulting pellet was resuspended in 5 ml of 0.6 mol/L potassium chloride, 30 mmol/L histidine buffer (pH 7.0) to solubilize actin and myosin and recentrifuged at 4° C at 45,000g for 30 minutes. The final pellet was resuspended in 25 mmol/L Tris buffer (pH 7.5) with 10 mmol/L magnesium chloride, 10 μg/ml bacitracin, 0.5 mmol/L dithiothreitol, and 0.2% bovine serum al-

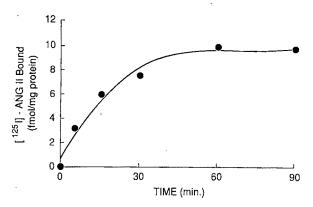


Fig. 1. Representative time to equilibrium study for incubation of vascular smooth-muscle receptor plasma membranes with 125 I-angiotensin II (ANG II). Plasma membranes were prepared from nonpregnant aorta and studied at 18° C. Specifically bound 125 I - angiotensin II is plotted as a function of incubation time; each point was determined in duplicate.

ranged from 80 µg/100 µl to 130 µg/100 µl as determined by modification of the Lowry method.18

Angiotensin II receptor binding assays were performed with 100 µl of membrane preparation in a total volume of 150 µl of the 25 mmol/L Tris buffer with 0.2% bovine serum albumin. Freshly prepared membranes were used in all experiments because binding was found to decrease with storage. Tyrosyl iodine 125labeled [5-L-isoleucine] angiotensin II (125 I - angiotensin II, New England Nuclear Corp., Boston, Mass.) was added in concentrations from 0.3 to 0.4 nmol/L. Unlabeled angiotensin II was added in increasing amounts from 10⁻¹¹ mol/L to 10⁻⁸ mol/L; the binding characteristics were determined from analysis of the displacement of labeled ligand with the methods of Baker et al.17 Nonspecific binding was determined by the addition of 10⁻⁵ mol/L angiotensin II. Specific binding of ¹²⁵I-angiotensin II reached equilibrium by 60 minutes of incubation for each artery studied (Fig. 1); therefore 60-minute incubations were used in all binding studies. Linearity of specific radioligand binding versus the amount of membrane protein incubated was confirmed (Fig. 2). Competitive binding studies were performed with 10^{-10} mol/L to 10^{-5} mol/L unlabeled angiotensin II, angiotensin III, angiotensin I, Sar¹, Thr⁸-angiotensin II, and arginine vasopressin (Sigma Chemical Co., St. Louis). Incubations for binding assays were performed at 18° C and were terminated by rapid addition of 4 ml of ice-cold 25 mmol/L Tris buffer with 0.2% bovine serum albumin. Bound and free ligands were then immediately separated by filtration through filters (Whatman GF/C, Whatman International Ltd., Maidstone, England) under vacuum, after which the filters were rinsed three times with buffer. After drying, radioactivity collected on the filter was determined with a scintillation counter (Packard Instruments, Downers Grove, Ill.) with an efficiency of 70% for 125 I. Binding

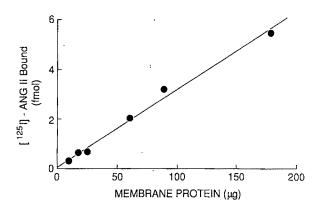


Fig. 2. Representative study of linearity of specific 125 I - angiotensin II (ANG II) binding versus amount of plasma membrane protein incubated for 60 minutes at 18° C. Plasma membranes were prepared from nonpregnant aorta. Each point was determined in duplicate.

paired arteries obtained from the same animal; all determinations were performed in duplicate.

The density of angiotensin II vascular smooth-muscle receptor (B_{max}) and the dissociation constant (K_d) were calculated from the specific binding data with a modification of the computer program LIGAND¹⁹ adapted for microcomputers by McPherson (Elsevier BIOSOFT, Cambridge, England).20 Hill coefficients were calculated from the same program.21

Plasma angiotensin II assay. Jugular venous blood (6 ml) was collected in chilled plastic syringes, immediately placed in cold tubes containing ethylenediaminetetraacetic acid and 60 µl of 0.125 mol/L 1,10phenanthroline, and centrifuged at 4° C; plasma was separated and stored at -20° C until the time of assay. The methods for extraction and the radioimmunoassay procedures have been previously published.9 The sensitivity of the assay was 1.3 pg per tube, and the recovery was $73\% \pm 2$ (mean \pm SEM). Cross-reactivity of the antiserum (titer 1:15,000) was 0.72% for angiotensin I, 22.6% for [Des-Asp¹]-angiotensin I, and 77.2% for [Des-Asp¹]-angiotensin II. Within-assay variability was determined from replicants (n = 6) of three plasma pools obtained from sheep and was 7.1%, 5.9%, and 5.6%. Between-assay variation was determined by assigning samples from these pools (n = 6) in each assay and was 10.4%, 9.2%, and 8.7%.

Statistical methods. Binding characteristics for different arteries and groups were compared with analysis of variance and Newman-Keuls test for multiple comparisons. Matched data were compared by using Student's t test. All data are presented as mean \pm SD unless otherwise noted.

Results

Plasma angiotensin II. Venous plasma concentrations of angiotensin II in the first group of ewes were

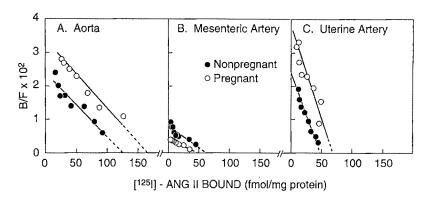


Fig. 3. Representative Scatchard plots of 125 I—angiotensin II (ANG II) binding to plasma membrane fractions prepared from aorta (A), mesenteric arteries (B), and uterine arteries (C) obtained from nonpregnant (solid circle) and pregnant (open circle) ewes. Ratio of bound to free $(B/F)^{125}$ I—angiotensin II is plotted as a function of specifically bound 125 I—angiotensin II. Each point was determined in duplicate.

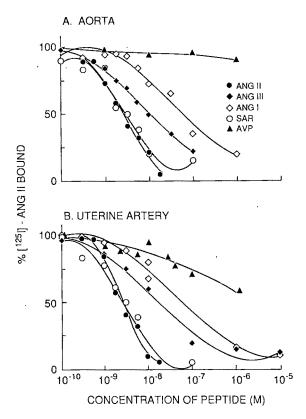


Fig. 4. Displacement curves for ¹²⁵I-angiotensin II (ANG II) binding to plasma membrane fractions from aorta (A) and uterine artery (B). Values are means of three to six experiments with each peptide, each performed in duplicate. SAR, [Sar³, Thr³]-angiotensin II; AVP, arginine vasopressin.

with nonpregnant (n = 6), 30 ± 7.6 pg/ml (0.03 nmol/L) and 6.7 ± 1.3 pg/ml (0.007 nmol/L) (p < 0.001), respectively. Similar results were obtained in the second group of ewes, that is, 29 ± 6.7 pg/ml (0.03 nmol/L) and 8.4 ± 1.7 pg/ml (0.008 nmol/L)

(p < 0.001), respectively, a 3.5-fold rise during pregnancy.

Angiotensin II receptors. LIGAND analysis of the displacement of 125 I—angiotensin II by unlabeled angiotensin II demonstrated a one-site model (p < 0.05, r > 0.90) and thus a single class of saturable binding sites. Representative Scatchard plots of specific binding data are presented in Fig. 3 for each of the arteries studied from nonpregnant and pregnant sheep. Specific binding at K_a was generally >80%; the vast majority of nonspecific binding was accounted for by binding of the radioligand to the filters. This was not altered by prewetting the filters with albumin-containing buffer. Hill coefficients ranged from 0.97 to 1.02, indicating that cooperativity was not involved in the binding of 125 I—angiotensin II to the vascular smooth-muscle receptor. 21

The ability of several angiotensin II analogues and the unrelated peptide hormone arginine vasopressin to displace ¹²⁵I—angiotensin II was examined. Representative displacement curves for vascular smooth muscle from aorta and uterine artery are illustrated in Fig. 4. The order of potency was saralasin = angiotensin II > angiotensin III > angiotensin I (inhibitory concentration of 50%: 5, 4, 18, and 80 nmol/L). Vasopressin did not displace ¹²³I—angiotensin II except at high concentrations. These findings are characteristic of binding to angiotensin II receptors.²²

In the first group of animals we examined the binding characteristics in two systemic arteries, the aorta and mesenteric arteries. Although $B_{\rm max}$ was more than twofold greater (p < 0.05) in the aorta as compared with the mesenteric artery in both nonpregnant (186 ± 29 fmol/mg protein vs 92 ± 21 fmol/mg protein) and pregnant (220 ± 46 fmol/mg protein) are greater was no difference between nonpregnant and pregnant receptor density for either vessel. The K_d for both systemic

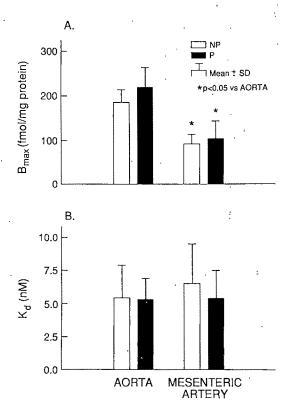
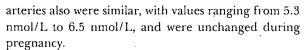


Fig. 5. Effect of pregnancy on angiotensin II receptor—binding density (B_{max}) (A) and dissociation constants (K_d) (B) in ovine aorta (n = 5) and mesenteric artery (n = 5). P, Pregnant; NP, nonpregnant.



In the second group of animals we compared the angiotensin II vascular smooth-muscle receptor in the systemic, that is, the mesenteric artery, and uterine arteries (Fig. 6). As in the first group there was no difference in B_{max} or K_d in the mesenteric artery of nonpregnant versus pregnant (78 ± 14 fmol/mg protein vs 61 ± 21 fmol/mg protein) ewes. Furthermore, these values were similar to those seen in the first group. Receptor density in the uterine artery also was not different in nonpregnant versus pregnant (59 \pm 20 fmol/mg protein vs 77 ± 20 fmol/mg protein), as was the K_d. Although the values for receptor density were similar to those seen in the mesenteric artery, the K_d in the mesenteric artery was 88% to 96% greater than that for nonpregnant or pregnant uterine artery vascular smooth-muscle receptor.

Comment

Regulation of the angiotensin II vascular smoothmuscle receptor during pregnancy remains an enigma; whether it is down-regulated and this accounts for the attenuated pressor responses to infused angiotensin II

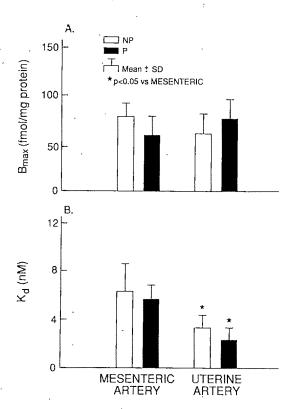


Fig. 6. Effect of pregnancy on angiotensin II receptor binding density (B_{nuc}) (A) and dissociation constants (K_d) (B) in ovine mesenteric (n = 5) and uterine (n = 5) artery. P, Pregnant; NP, nonpregnant.

seen in pregnant women² and several other species⁴⁻⁷ is controversial. This controversy emanates from studies in women and animals from which it has been suggested that down-regulation of the angiotensin II vascular smooth-muscle receptor either does14,16 or does not8,13 occur during normal pregnancy. In our investigation we identified a single class of high-affinity angiotensin II receptors in vascular smooth muscle of three different arteries obtained from normal pregnant and nonpregnant ewes with binding characteristics similar to those reported by others.²² More specifically we provide evidence for the first time that the B_{max} in systemic arteries does not decrease in spite of a threefold to fourfold increase in circulating levels of angiotensin II during ovine pregnancy, and the affinity is also unchanged. Thus it is unlikely that the attenuated pressor responses observed during ovine pregnancy,4.5 which are strikingly similar to that seen in human pregnancy,2 can be explained by these mechanisms. Furthermore our observation of an even greater refractoriness to the vasoconstrictor effects of angiotensin II in the uteroplacental vascular bed as compared with the systemic vasculature10,11 also cannot be explained by a decrease in the density or affinity of the uterine artery angiotensin II vascular smooth-muscle receptor. It is note1646 Mackanjee et al.

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worthy that the K_d for each of these vessels was similar to the plasma concentration of angiotensin II necessary to elicit a 50% rise in mean arterial pressure in pregnant and nonpregnant ewes^{4, 9} (i.e., 1 to 2×10^{-9} mol/L), providing evidence that the binding sites examined are the biologically active receptors.

It has repeatedly been shown in pregnant adult animals that the binding capacity of the angiotensin II vascular smooth-muscle receptor responds in a reciprocal manner to changes in plasma angiotensin II.22, 23 Thus it would have been anticipated that down-regulation or increased occupancy of the angiotensin II vascular smooth-muscle receptor occurs during ovine pregnancy because plasma angiotensin II levels are normally increased. 8,9 Support for this conclusion, however, has previously been obtained primarily from studies of nonvascular smooth muscle15, 16 such as myometrium, which in nonpregnant animals responds to changes in plasma angiotensin II in a manner similar to vascular smooth-muscle receptor.28, 24 Although decreases in myometrial receptor-binding capacity were observed in the rat15 and sheep16 late in pregnancy, Paller⁷ observed no change in the angiotensin II-binding capacity in bladder smooth muscle of pregnant rats. Because the pregnant rat was used in the study of both tissues, it is evident that differences in receptor modulation in response to alterations in plasma angiotensin II exist in different types of smooth muscle. Similar observations have been made between the vascular smooth-muscle receptor and the adrenal glomerulosa, the latter of which exhibits parallel rather than reciprocal changes in binding capacity in the presence of elevated plasma angiotensin II.23, 24 There also may be important differences between species in the regulation of these nonvascular angiotensin II receptors. Therefore it might be suggested that the observations of Baker et al.25 of down-regulation of platelet angiotensin II receptors in gravid women also may not reflect alterations in vascular smooth-muscle receptor. Thus only the data of Brown and Venuto,26 who reported decreases in receptor density in the mesenteric artery of pregnant rabbits, can be directly compared with our data. Because we observed no pregnancy-associated differences in receptor-binding characteristics in the three arteries studied, it is possible that the discrepancy between our data and those of Brown and Venuto reflect species differences in receptor regulation.

The observed association between decreased systemic pressor responses and an unaltered binding capacity for angiotensin II vascular smooth-muscle receptor in the presence of elevated plasma angiotensin II levels in pregnancy may be novel. It is possible that in the sheep the vascular smooth-muscle receptor does not respond to alterations in plasma angiotensin II. However, we have shown in nonpregnant ewes that in-

creased responsiveness to infused angiotensin II occurs after volume expansion,8 and Blair-West et al.5 have reported decreased responses in salt-depleted non-pregnant and pregnant ewes. Furthermore, treatment with angiotensin-converting enzyme inhibitors increases the pressor response to infused angiotensin II in pregnant ewes. Although these data suggest that the angiotensin II vascular smooth-muscle receptor in sheep is responsive to alterations in plasma levels of the peptide, this has not been documented in direct studies of the vascular smooth-muscle receptor. Alternatively, the plasma levels of angiotensin II normally achieved in ovine pregnancy may not be sufficient to down-regulate the receptor. This too has not been studied.

If the angiotensin II vascular smooth-muscle receptor is not down-regulated in ovine pregnancy, how is receptor-binding capacity maintained in the presence of elevated plasma angiotensin II? Schiffrin et al.27,28 have reported that in adult male rats either the mesenteric angiotensin II vascular smooth-muscle receptor is up-regulated or down-regulation is prevented when elevation in plasma angiotensin II concentrations occur concomitantly with increased plasma levels of aldosterone or deoxycorticosterone or both. This may be applicable to the condition of pregnancy because elevated plasma levels of these mineralocorticoids occur in normal pregnancy,1,29 likely reflecting the relative underfilling associated with this condition and thus the need to retain sodium and water. It may be suggested that during pregnancy there is a need to maintain not only vascular tone in the presence of substantial systemic vasodilation but also vascular volume, which permits the increases in cardiac output that occur during pregnancy.3 Theoretically both can be achieved by activation of the renin-angiotensin system, which would result in increased secretion of mineralocorticoids and up-regulation or maintenance of the angiotensin II vascular smooth-muscle receptor by mechanisms not yet understood.

Alternative explanations for the maintenance of angiotensin II vascular smooth-muscle receptor binding in the face of elevated plasma angiotensin II levels may reside in other hormonal changes that occur during normal pregnancy. Plasma estrogen concentrations are increased during ovine pregnancy,30 primarily reflecting placental production. In studies of cycling rats, myometrial angiotensin II receptor binding increased fourfold during proestrus, when the plasma estrogen/progesterone ratio was high, and decreased during diestrus, when the estrogen/progesterone ratio was low.31 In neither instance was receptor affinity altered. Ovariectomy resulted in a decrease in myometrial angiotensin II receptors that was reversed by estrogen administration.8 Furthermore, progesterone inhibited this effect of estrogen. Similarly, estrogen administered

to pregnant rats caused a threefold increase in plasma angiotensin II, but no change in placental angiotensin II binding density, and progesterone-induced decreases in placental binding were inhibited by simultaneous administration of estrogen.32 Thus estrogen may participate in up-regulating or maintaining angiotensin II receptor binding capacity. We have observed in nonpregnant sheep that acute short- or long-term estrogen treatment increases plasma angiotensin II38 and is associated with attenuated pressor responses to infused angiotensin II.33,34 However, we have not yet examined the angiotensin II vascular smooth-muscle receptor to determine whether observations similar to those in the pregnant rat can be made in the ewe.

If the angiotensin II vascular smooth-muscle receptor is not down-regulated in pregnancy, another explanation must be sought for the refractoriness to this vasoconstrictor during pregnancy. In pregnant ewes,35 human beings, and several other species36, 37 there is evidence of increased production of vasodilator prostanoids, and vasoconstrictor responses to infused angiotensin II are increased after cyclooxygenase inhibition.³⁸ Furthermore, prostacyclin production by uterine arteries from pregnant sheep³⁹ is enhanced by angiotensin II through a receptor-mediated mechanism. Thus vasodilator prostanoids may antagonize the effects of angiotensin II, especially in the uteroplacental vascular bed. More recently we have reported that protein kinase C activity in the uterine artery decreases during ovine pregnancy and may be related to an increase in the estrogen/progesterone ratio.30 This too could account in part for the attenuated vasoconstrictor responses. Moreover, because angiotensin II stimulates protein kinase C activity, this effect of estrogen may explain how estrogen can maintain angiotensin II receptor-binding capacity in the face of increased plasma levels of angiotensin II32 and yet not be associated with an increase in vasoconstrictor responses.33,34 This, however, requires further study. Finally, at least two angiotensin II receptor subtypes have recently been described.40 It is possible that during pregnancy the maintenance of vascular smooth-muscle receptor binding capacity reflects increased expression of the AT2 nonvascular receptor in vascular smooth muscle, the action of which is not mediated through calcium and is not involved with contraction, and a decreased expression of the AT₁ vascular receptor. This also would explain the attenuated vascular responses to infused angiotensin II in the absence of down-regulation.

In summary, we have shown that during normal ovine pregnancy there is no evidence of decreased angiotensin II receptor-binding capacity or affinity in the vascular smooth muscle in three different arteries, suggesting that the attenuated vascular responses to this peptide in the systemic and uteroplacental vascular beds during pregnancy do not reflect receptor downregulation. It is more likely that this is caused by a series of rather complex interactions that may include the effects of the steroid hormones on the receptor itself, receptor signaling, the antagonistic effects of vasodilator prostanoids, and possibly the increased expression of the "nonvascular" receptor subtype in vascular smooth muscle.

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Regulation of corticotropin responsiveness in human fetal adrenal cells

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The human fetal adrenal gland exhibits a high rate of steroidogenesis during fetal development and produces the majority of steroids used by the placenta for estrogen synthesis. Corticotropin appears to be the principal hormonal regulator of steroidogenesis in the fetal adrenal gland. However, little is known concerning the regulation of corticotropin receptors. In this study we examined the long-term regulation of corticotropin responsiveness as measured by the ability of human fetal adrenal gland cells to produce cyclic adenosine monophosphate after corticotropin treatment for 3 hours. We also examined the regulation of corticotropin receptors as determined by iodine 125-labeled corticotropin binding to fetal adrenal cells. Fetal adrenal glands were obtained from second-trimester abortuses. The two distinct zones of the fetal adrenal gland, the definitive zone and the fetal zone, were separated and the tissue mechanically dispersed. Freshly isolated cells responded to corticotropin with a sevenfold to tenfold increase in the production of cyclic adenosine monophosphate, indicating a functional corticotropin receptor-adenylate cyclase coupling. However, when either fetal zone or definitive zone cells were grown and passed in monolayer culture, corticotropin stimulation of cyclic adenosine monophosphate production dropped to only twofold. The loss of corticotropin stimulation of cyclic adenosine monophosphate production occurred with a loss of the steroid-metabolizing enzyme 17α-hydroxylase (P-450_{17a}). Because P-450₁₇₄ expression can be stimulated after treatment of fetal adrenal gland cells with corticotropin or forskolin, we attempted to increase the ability of corticotropin to stimulate cyclic adenosine monophosphate production in a similar manner. After cells were pretreated with corticotropin (0.1 to 100 nmol/L) or forskolin (0.1 to 100 μmol/L) for 4 days, their ability to produce cyclic adenosine monophosphate in response to corticotropin was examined. Pretreatment with both corticotropin and forskolin caused a dosedependent increase in the ability of corticotropin to stimulate the production of cyclic adenosine monophosphate. Cells stimulated with corticotropin after pretreatment with forskolin exhibited a 35- to 50-fold increase in cyclic adenosine monophosphate production compared with ncntreated cells (=twofold). Corticotropin pretreatment increased responsiveness to a lesser extent than forskolin pretreatment. The increase in corticotropin responsiveness occurred along with ar induction of P-450_{17a} enzyme levels. The effect of pretreatment with corticotropin and forskolin on the binding of iodine 125-labeled corticotropin to definitive zone cells was also investigated. Corticotropin pretreatment increased corticotropin receptor binding 2.8 times; forskolin pretreatment increased corticotropin binding by seven times. These data indicate that corticotropin responsiveness and corticotropin receptors in human fetal adrenal cells are regulated by a cyclic adenosine monophosphate-dependent mechanism. This observation is similar to findings in bovine and ovine adrenal cells where the number of corticotropin receptors is increased by corticotropin or cyclic adenosine monophosphate treatment. (AM J OBSTET GYNECOL 1991;165:1649-54.)

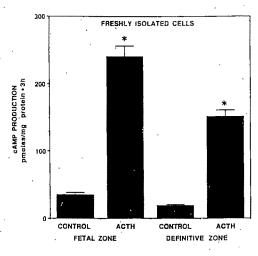
Key words: Fetal adrenal gland, definitive zone cells, fetal zone cells, cell culture, corticotropin responsiveness

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The human fetal adrenal gland is characterized by hyperplasia and high levels of steroid production. The diminished adrenal gland size in anencephalic fetuses and reduced fetal adrenal gland steroidogenesis after fetal exposure to exogenous glucocorticoid suggest that fetal pituitary corticotropin is one factor essential to normal adrenal gland development and function. A number of laboratories have made use of in vitro models to determine the mechanism by which corticotropin controls human fetal adrenal gland function. Dispersed



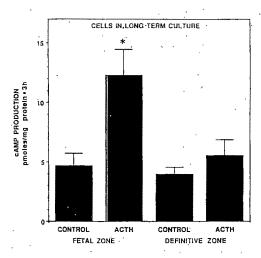


Fig. 1. Corticotropin (ACTH) responsiveness; freshly isolated cells versus cells in long-term monolayer culture. Definitive zone and fetal zone cells were treated for 3 hours with corticotropin (100 nmol/L). Medium content of cAMP was determined by radioimmunoassay. Values represent mean \pm SD for four different dishes of cells. Asterisk, Values significantly different from respective control value (p > 0.001).

cells have been useful for examination of the acute effects of corticotropin treatment.^{3,4} Studies of primary cultures of human fetal adrenal cells have been useful in defining the chronic role of corticotropin in regulating adrenal cell differentiation. Fetal adrenal cells grown in culture exhibit decreased steroid hormone production as well as reduced levels of steroid-metabolizing enzymes. Corticotropin treatment of these cells increases steroidogenesis and the expression of steroid-metabolizing enzymes.^{5,9} Thus both in vivo and in vitro evidence suggests a key role of corticotropin in maintaining fetal adrenal cell steroidogenic capacity.

The ability of adrenal cells to respond to corticotropin treatment depends on the ability of this peptide to stimulate production of cyclic adenosine monophosphate (cAMP). Recent evidence obtained with adult ovine and bovine adrenal cells suggests that corticotropin responsiveness is regulated in a cAMP-dependent manner.10.11 However, little is known about corticotropin receptor regulation in human fetal adrenal cells. Previous reports have shown that human fetal adrenal cells do not undergo desensitization after corticotropin treatment but actually show increased responsiveness after long-term corticotropin stimulation.9 In this study we examined the effects of chronic treatment with corticotropin and forskolin on corticotropin responsiveness. Our data give strong support to the hypothesis that corticotropin is able to increase expression of its own receptor level in human fetal adrenal cells.

Material and methods

Cell preparation. Fetal adrenal glands were obtained from second-trimester (12 to 18 weeks' gestation) human abortuses delivered electively by dilation and ex-

traction. The tissues were obtained under the auspices of the Donors Anatomical Gift Act of the State of Texas after obtaining written consent from the women having the abortions. The consent form and the experimental protocol were approved by the Human Research Review Committee of the University of Texas Southwestern Medical Center at Dallas.

After separation of the fetal and the definitive zones, the adrenal glands were minced finely and placed in Hanks' Ca2+- and Mg2+-free medium that contained N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer (25 mmol/L, pH 7.4). The tissue fragments were dispersed by repeated aspiration and expulsion with a 5 ml plastic pipette at room temperature. The dispersed tissue was filtered through gauze to remove the remaining fragment aggregates, and the filtrate was centrifuged at 800g for 10 minutes. For experiments with freshly dispersed cells, the pellet was resuspended in Ham's F-12-Dulbecco's modified Eagle's medium and treated immediately. For monolayer culture the cells were suspended in Ham's F-12-Dulbecco's modified Eagle's medium containing 5% horse serum, 5% fetal bovine serum, and 1% antibiotics-antimycotics and plated at a density of 5×10^6 cells per 75 cm² flask. The cells were allowed to attach to the plastic dish for 24 hours; thereafter the medium (20 ml per dish) was replaced every 48 hours. The cells were maintained in this medium in a humidified atmosphere of carbon dioxide (5%) and air (95%) at 37° C. At confluency (7 to 10 days) the cells were subcultured with a trypsin-ethylenediaminetetraacetic acid solution (Sigma Chemical Co., St. Louis) onto five flasks (75 cm²) and allowed to grow to confluency, when they were trypsinized, placed in vials, and stored frozen in

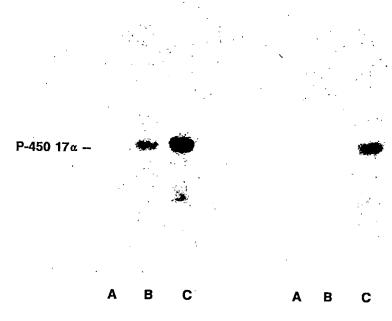


Fig. 2. Regulation of P-450_{17a} enzyme expression in definitive zone (right) and fetal zone (left) cells maintained in culture for 3 to 4 weeks. Cells were then treated with either corticotropin (10 nmol/L) or forskolin (10 μ mol/L). Immunoblotting analysis was carried out on 100. μ g of total cell protein for P-450_{17a}. A, Control; B, corticotropin; C, forskolin.

liquid nitrogen. For each experiment one vial of cells was unthawed and cultured in 12-well dishes. Cells were maintained in culture for a total of 3 to 4 weeks before the experiments.

Immunoblotting. After treatments, cells were washed with 0.9% sodium chloride solution and removed in a small volume of lysing buffer as previously. described.12 One-dimensional electrophoresis was performed in a 12% polyacrylamide gel with a gel system (Precast, Amersham, Arlington Heights, Ill.). Sample buffer contained 0.05 mol/L Tris (pH 6.8), 2% sodium dodecyl sulfate, 10% glycerol, and 8 mmol/L ethylenediaminetetraacetic acid; samples were loaded after boiling for 3 minutes in the presence of 2-mercaptoethanol. Electrophoresis was carried out at 30 V at room temperature. Proteins were transferred to a nitrocellulose paper in an electrophoresis unit (Transphor, Hoefer Scientific Instruments, San Francisco) at 100 V for 1 hour at 2° to 4° C in a buffer containing 20% methanol, 20 mmol/L Tris, and 150 mmol/L glycine. The nitrocellulose membrane was incubated for 15 minutes at room temperature in a blocking buffer containing 10 mmol/L Tris, pH 7.4, 0.15 mol/L sodium chloride, 0.2% Nonidet P-40, and 0.5% dry milk and placed for 2 hours in the same buffer containing polyclonal antibodies (10 µg immunoglobulin G per milliliter) against human placental 3β-hydroxysteroid dehydrogenase or pig testis P-450_{17a}. Membrane blots were washed in the same buffer without antibody for 15 minutes and then incubated in the blocking buffer containing approximately 106 cpm/ml of iodine 125labeled protein A (ICN Biochemicals, Irvine, Calif.) for

30 minutes. Finally the nitrocellulose membrane was washed in blocking buffer for 1 hour before exposure to photographic film. All incubations and washings were performed at room temperature.

Cyclic adenosine monophosphate determinations. The measurement of cAMP was quantified in the culture medium with radioimmunoassay kits (Advanced Magnetics, Cambridge, Mass.). For each assay the acetylated procedure for increased sensitivity was used. Standard curves for cAMP were constructed in the incubation medium and acetylated therein. The preparation of standards in incubation medium increased assay sensitivity. Extraction of the medium was not necessary. Statistical differences were determined with one-way analysis of variance and Newman-Keuls multiple comparison analysis.

Corticotropin binding. Experiments were performed on 12-well plates containing approximately 105 cells per well. For binding studies the human fetal adrenal cells were treated in a medium containing 2% Ultraser G (IBF Biotechnics, Villeneuve-la-Garenne, France). Culture with this medium supplement was found to increase corticotropin binding to human fetal adrenal cells. Cells were washed four times with medium composed of Dulbecco's modified Eagle's-F-12 medium plus HEPES (15 mmol/L, pH 7.4). Binding assays were carried out for 1 hour at room temperature as described previously. 10-12 In brief, 0.5 ml of binding medium (Dulbecco's modified Eagle's-F-12 medium; 0.5% bovine serum albumin; 0.1% bacitracin; 15 mmol/L HEPES containing 125 I-tyrosine 23-human corticotropin 1-39 (\approx 200,000 cpm per well \approx 60 fmol per

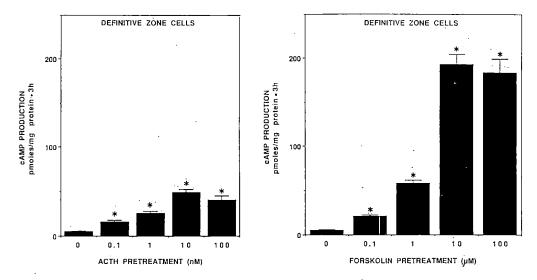


Fig. 3. Regulation of corticotropin (ACTH) responsiveness. Definitive zone cells were maintained in culture for three to four wells, then treated with indicated concentration of corticotropin or forskolin for 4 days. Cells were rinsed and treated with corticotropin (10 nmol/L) for 3 hours. Medium content of cAMP was determined by radioimmunoassay. Values represent mean \pm SD for four different dishes of cells. Asterish, Values were significantly different from controls (p > 0.001).

well) was used. ¹²⁵Í-tyrosine 23 human corticotropin 1-39 (specific activity of 1900 Gi/mmol or 70 TBq/mmol) was obtained (Amersham, Arlington Heights, Ill.). The nonspecific binding was determined with unlabeled corticotropin 1-24 (1 × 10⁻⁶ mol/L) and represented 5% to 10% of the total bound. At the end of incubation plates were placed on ice and cells were washed four times with medium (Dulbecco's modified Eagle's–F-12 medium containing HEPES, 15 mmol/L, pH 7.4) at 4° C. The cell layer was then lysed in 0.4% deoxycholate, and radioactive lysates were counted in a gamma radiation spectrometer.

Results

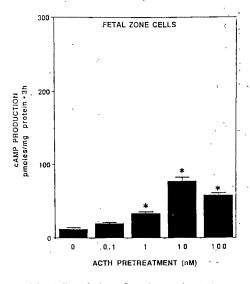
To examine human fetal adrenal cell responsiveness to corticotropin we examined the ability of both definitive zone and fetal zone cells to produce cAMP after treatment with corticotropin for 3 hours. Freshly isolated definitive zone and fetal zone cells responded to corticotropin (100 nmol/L) treatment with a 7- to 10-fold increase in cAMP production (Fig. 1). When these cells were maintained in culture for 3 to 4 weeks there was a decline in response to corticotropin. Specifically, definitive zone and fetal zone cells not only decreased the amount of cAMP produced after corticotropin treatment but responded with only a 1.5- to 2.5-fold increase of cAMP production above base levels.

The decline in corticotropin responsiveness appeared to be similar to the loss of steroid-metabolizing enzymes seen in these cells maintained in long-term culture. Definitive zone and fetal zone cells maintained for 3 to 4 weeks in culture exhibited nondetectable levels of immunoreactive P-450_{17a} (Fig. 2). Treatment

of these cells with corticotropin (10 nmol/L) or forskolin (10 μ mol/L) for 4 days caused expression of P-450_{17a}. The induction of enzyme expression after treatment with forskolin was greater in both fetal zone and definitive zone than when corticotropin was used.

To see whether responsiveness could be regained in a manner similar to that with P-450_{17α}, we treated fetal zone and definitive zone cells for 4 days with corticotropin (0.1 to 100 nmol/L). After treatment, cells were washed four times to remove residual corticotropin and then treated with corticotropin (100 nmol/L) for 3 hours. Fetal zone and definitive zone cells pretreated with corticotropin were more responsive to acute stimulation of cAMP production (Figs. 3 and 4). Corticotropin pretreatment caused a concentration-dependent increase in the ability of corticotropin to stimulate cAMP production. Significant effects of corticotropin pretreatment were observed at a concentration of 1 nmol/L. Forskolin pretreatment also increased definitive zone and fetal zone responsiveness to corticotropin (Figs. 3 and 4). The ability of forskolin pretreatment to increase corticotropin responsiveness was more pronounced than that seen after corticotropin pretreatment.

We examined the effects of chronic treatment with corticotropin or forskolin on corticotropin receptors (Fig. 5). Definitive zone cells were treated for 4 days with corticotropin (10 nmol/L) or forskolin (10 µmol/L). Relative changes in corticotropin receptors were determined by examining binding of ¹²⁵I-tyrosine 23 human corticotropin 1-39. Binding increased 2.8 times after corticotropin pretreatment and seven times after forskolin pretreatment.



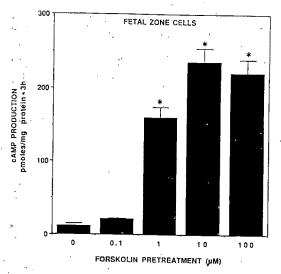


Fig. 4. Regulation of corticotropin (ACTH) responsiveness. Fetal zone cells were maintained in culture for 3 to 4 weeks, then treated with indicated concentrations of corticotropin or forskolin for 4 days. Cells were rinsed and stimulated with corticotropin (100 nmol/L) for 3 hours. Medium content of cAMP was determined by radioimmunoassay. Values represent mean \pm SD for four different dishes of cells. Asterisk, Values significantly different from controls (p > 0.001).

Comment

This report constitutes the first examination of the regulation of human fetal adrenal cell responsiveness to corticotropin. Our findings demonstrate that responsiveness to corticotropin is lost when both fetal zone and definitive zone cells are maintained in long-term culture. Responsiveness was, however, regained when the cells were treated with corticotropin or forskolin. These data support the hypothesis that corticotropin receptors and responsiveness, like the induction of steroid-metabolizing enzymes, are regulated in a cAMP-dependent manner.

Human fetal adrenal cells have been extensively used to examine the regulation of steroid hormone production.³⁻⁹ With time in culture these cells exhibit a decline in base levels of steroid production and expression of steroid-metabolizing enzymes. The loss of these functions is believed to be caused by the removal of the cells from a source of corticotropin. Treatment with corticotropin will stimulate steroidogenesis and the expression of several steroid-metabolizing enzymes.⁵⁻⁹ Various detailed studies have demonstrated that steroid production and the enzymes necessary for steroidogenesis are regulated in a cAMP-dependent manner.

In addition to the loss of steroid-metabolizing enzymes, bovine adrenal cells in culture have been shown to exhibit a reduced ability to respond to corticotropin.¹³ The simultaneous loss of both corticotropin response and steroidogenic enzymes suggests that these events might be regulated in a similar manner. To address this question definitive zone and fetal zone cells were grown for 3 to 4 weeks in monolayer cell culture,

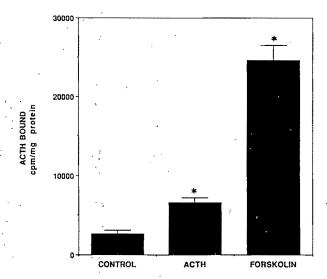


Fig. 5. Regulation of 125 I-corticotropin (ACTH) binding to definitive zone cells maintained in culture for 3 to 4 weeks, then treated with corticotropin (10 nmol/L) or forskolin (10 μ mol/L) for 4 days. Cells were rinsed and 125 I-corticotropin binding examined. Values represent mean \pm SD for specific binding to four separate dishes of cells. For corticotropin binding studies cell culture medium was supplemented with Ultraser G. This medium supplement was found to increase 125 I-corticotropin binding. Asterisk, Values significantly different from control (p > 0.001).

after which corticotropin stimulation of cAMP production was examined. In agreement with reports in which bovine adrenal cells were used, human fetal adrenal cells maintained in long-term cell culture exhib-

ited a reduced response to corticotropin. To determine whether responsiveness was regulated in a manner similar to that of steroid-metabolizing enzymes we treated cells for 4 days with either corticotropin or forskolin. The expression of P-450_{17α} was markedly enhanced by both factors. In addition the responsiveness to corticotropin was increased by both corticotropin and forskolin. This effect is similar to that seen in bovine adrenocortical cells where corticotropin treatment can increase the ability of the cells to respond to corticotropin. ¹⁰

The mechanism by which corticotropin stimulates cyclic adenosine monophosphate production is complex and relies on the expression of corticotropin receptors, guanosine triphosphate-binding proteins, and adenylate cyclase. To determine whether corticotropin receptor expression was increased in human fetal adrenal cells, we examined the binding of 125 I-corticotropin to definitive zone cells. Both corticotropin and forskolin increased binding of 125I-corticotropin (Fig. 5). The ability of corticotropin to increase corticotropin receptor expression has also been demonstrated in cultures of adult bovine and ovine adrenal cells.10,11 Corticotropin also appears to increase the expression of guanosine triphosphate-binding proteins in bovine adrenal cells.14 Thus corticotropin treatment of adrenal cells may act to up-regulate the corticotropin receptor and coupling system.

These data support the hypothesis that corticotropin is a positive regulator of its own responsiveness. The positive effect of corticotropin on its own receptor may have physiologic significance. In the fetal adrenal gland corticotropin receptor expression could be regulated by plasma corticotropin levels. Previously we have shown that corticotropin activation of adenylate cyclase is low in the anencephalic fetal adrenal gland where circulating levels of corticotropin are low. 15 In the ovine and goat fetus the ability of fetal adrenal cells to respond to corticotropin appears to parallel the fetal plasma levels of corticotropin. 16-18 Such data suggest that enhanced glucocorticoid levels observed in response to chronically elevated plasma corticotropin are related to at least two effects: (1) a positive trophic effect on the expression of several steroid-metabolizing enzymes and (2) a positive trophic effect of corticotropin on its own receptor.

We appreciate the technical assistance of Nora Cline.

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The metabolic clearance rate of epinephrine in the fetus of the diabetic ewe

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Delayed organ maturation is a characteristic of the fetus of the diabetic mother with poor glucose control. We hypothesized that the ovine fetal catecholamine maturation sequence would be delayed in the fetus of the diabetic ewe. Twelve pregnant ewes were rendered glucose intolerant by the administration of streptozocin at 85 to 95 days' gestation. Maternal diabetic status was verified with fasting blood glucose assessments. The fetal metabolic clearance rate of epinephrine was determined at 126 to 140 days' gestation by the constant infusion of 0.1 µg epinephrine per kilogram estimated feta weight per minute. Fetal arterial blood gas values, lactate, glucose, and insulin levels were measured before infusion; after 20, 30, and 40 minutes of epinephrine infusion; and 15, 30, and 60 minutes after cessation of infusion. Fetal plasma glucose rose significantly from a control level of 42.1 ± 8.2 to 64.6 ± 5.9 mg/dl during the epinephrine infusion (p < 0.05). Fetal insulin levels increased from a baseline of 9.2 \pm 2.6 μ IU/ml to 32.4 \pm 18.0 in the recovery period (p < 0.05), and lactate levels similarly rose from 36.4 \pm 4.8 to 52.2 \pm 8.2 mg/dl (p < 0.05). The plasma epinephrine production rates did not vary significantly between fetuses <135 and >135 days' gestation (15.4 \pm 1.5 vs 13.9 \pm 2.0 ng/min, p > 0.5). The fetal metabolic clearance rate of epinephrine at early gestations (<135 days) was similar to that of the fetus of the nondiabetic ewe (35.3 \pm 3.4 vs 28.0 \pm 4.3 ml/min/kg, p > 0.2). However, at later gestations (>135 days) the fetus of the diabetic ewe did not have the increase in the metabolic clearance rate previously published for the control fetus (32.0 \pm 4.6 vs 133.7 \pm 41.7 ml/min/kg, p < 0.05). These data appear to indicate an absence in the maturation of the metabolic clearance rate of epinephrine in the fetus of the diabetic ewe. The observed alteration in the metabolic clearance rate of epinephrine of the fetus of the diabetic ewe could potentially impair the response to stress. (AM J OBSTET GYNECOL 1991;165:1655-60.)

Key words: Ovine, diabetes, fetus, catecholamines, metabolic clearance rate

There is a well-defined catecholamine maturation sequence in the ovine fetus. Comline and Silver¹ in 1966 stimulated the adrenal glands of fetal lambs and showed that before 80 days' gestation dopamine is the principal catecholamine secreted. Early in the third trimester norepinephrine appeared in response to adrenal stimulation, and during the last 20 days of gestation epinephrine replaced norepinephrine as the dominant stress catecholamine. The ovine fetal adrenal gland increases in size and weight as the fetus matures² and undergoes maturation before the completion of gestation.¹

It has previously been reported that the plasma epinephrine concentration decreases as gestation advances in the ovine fetus.³ The production rate of epinephrine is constant throughout gestation in this species.⁴ The

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observed reduction in plasma epinephrine concentration is a secondary effect of an increase in the epinephrine metabolic clearance rate, which occurs at approximately 134 days' gestation.⁴

Delayed organ maturation is a characteristic of the fetus of the diabetic mother with poor glucose control. We hypothesized that the characteristic fetal epinephrine maturation sequence would be delayed in the fetus of the diabetic eye.

Material and methods

Twelve pregnant Western-bred ewes of known gestational age were rendered glucose intolerant by the intravenous administration of streptozocin (The Upjohn Co., Kalamazoo, Mich.) on two separate occasions, 4 days apart, commencing at 85 to 90 days' gestation. After an overnight fast, streptozocin was administered to the ewe at a dose of 50 mg/kg maternal weight. Immediately before injection, the streptozocin was reconstituted in 50 ml sterile 0.9% sodium chloride and then infused over 3 to 4 minutes through a maternal peripheral hind limb vein. To ensure active secretion of insulin and maximal degranulation of the pancreatic β-cells, the streptozocin was injected while the animals

were feeding. Maternal carbohydrate tolerance was assessed by serial fasting plasma glucose testing before and 4 to 6 weeks after streptozocin administration.

The ewes were transferred to the Animal Care Center at our institution at 112 to 120 days' gestation and acclimated for a minimum of 7 days before surgery. A diet of pelleted alfalfa and hay was fed to the ewes. The animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals' in the Animal Care Center of the University of Texas Medical School at Houston. The experimental protocol was approved by our institutional Animal Welfare Committee.

At 120 to 127 days' gestation, while the ewes were under general anesthesia, polyvinyl catheters were placed in the fetal dorsal aorta and vena cava through a dorsal pedal artery and vein. Catheters were also placed in the amniotic cavity and in a maternal lateral femoral circumflex artery and vein. The animals were allowed to recover for 5 to 7 days after surgery, at which time normal maternal and fetal arterial blood gas values were observed.

To evaluate the basal metabolic clearance rate of epinephrine, the experimental protocol consisted of a 10minute control period, a 40-minute intravenous fetal infusion of epinephrine (lot 75F-0226, Sigma Chemical Co., St. Louis), and a 60-minute recovery period. Maternal and fetal mean arterial pressure (MAP), fetal venous pressure, and amniotic cavity pressure were recorded continuously with a physiologic recorder (model R611, Beckman Instruments, Inc., Schiller Park, Ill.) and transducers (Statham model P23Db, Spectramed, Oxnard, Calif.). Maternal heart rate and fetal heart rate (FHR) were calculated from the arterial pulse every 5 minutes. Fetal arterial blood samples were collected for blood gas analysis and catecholamine, glucose, insulin, and lactate determinations at the beginning and end of the 10-minute control period; at 20, 30, and 40 minutes during the epinephrine infusion; and at 15, 30, and 60 minutes of recovery.

Epinephrine concentrations were measured in the infusate at the beginning and end of each infusion to detect accumulation of oxidative metabolites. The metabolic clearance rate was calculated from these measured epinephrine levels in the infusate. The infusate was prepared within 25 minutes of infusion from epinephrine standard dissolved at 0.9% normal saline solution with additional ethylene glycol-glutathione preservative. The epinephrine (0.1 μ g/kg estimated fetal weight) was infused at a rate of 0.1 ml/min into the fetal inferior vena cava by a pump (Harvard Apparatus Co., Millis, Mass.).

Fetal arterial blood gases were measured with a blood gas analyzer (model 158, Dow Corning Corp., Medfield, Mass.) at 39° C. The maternal and fetal plasma

glucose levels were assessed with a glucose analyzer (model 23A, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). Plasma insulin, levels were assessed with a specific radioimmunoassay (ICN Micromedic Systems, Horsham, Pa.), and lactate levels were measured enzymatically (Sigma Diagnostics, St. Louis). Catecholamine samples were collected in chilled syringes, placed in iced tubes containing ethylene glycol-reduced glutathione, and centrifuged. The plasma was removed and stored in a -70° C freezer before assay. Plasma catecholamine levels were determined by electrochemical detection with a glassy-carbon electrode (model 460 electrochemical detector, Waters Chromatography Div., Millipore Corp., Milford, Mass.) after separation on a high-pressure liquid chromatography C18 column as previously described.6 The assay sensitivity was 1 to 2 pg/ml for epinephrine with an interassay variation of 7% and an intraassay variation of

By means of the constant infusion method, the metabolic clearance rate of epinephrine was determined by the formula of Tait and Burnstein⁷:

$$MCR = P/(Css - Cb)$$

where MCR is the metabolic clearance rate, P is the production rate or amount infused, Css is the steadystate plasma epinephrine levels, and Cb is the baseline plasma epinephrine level. All values are corrected for body weight. The constant infusion method requires two assumptions to be made because there is no isotope labeling of the infused compound. First, steady-state levels of the compound under investigation must be achieved in all compartments. This is presumed when the plasma concentration reaches a steady state. Second, it is assumed that the dosage of the infused compound does not alter basal homeostasis or metabolic clearance. In preliminary studies significant alterations in heart rate, MAP, and arterial pH were not observed with a dose of 0.1 µg epinephrine per kilogram of weight.

Statistical analysis was performed with the Student t test and analysis of variance. Logarithmic transformation [Log(x + I)] was used when variances were not equal. All data are mean \pm SEM. A probability of p < 0.05 was chosen to represent statistical significance.

Results

To document the induction of maternal diabetes, serial fasting plasma glucose assessments were performed. After streptozocin administration, the fasting venous plasma glucose values of the 12 ewes rose significantly from a pretreatment level of 78.1 ± 7.2 to 108.2 ± 6.1 mg/dl 4 to 6 weeks later, p < 0.05. Fasting maternal glucose levels were conducted on five nontreated late-gestation ewes housed in the Animal Care

Center at the time of our experimentation. These control plasma glucose levels were $71.4 \pm 7.1 \text{ mg/dl}$, demonstrating no tendency toward carbohydrate intolerance with advancing gestation in the ewe. We believe these results confirm the presence of diabetes in the streptozocin-treated ewes.

There was no significant alteration in FHR and MAP from control values during the period of the epinephrine infusion or recovery (Fig. 1). The baseline FHR was 162.9 ± 5.2 beats/min, which increased nonsignificantly at the completion of the infusion and throughout the recovery period to 169.3 ± 5.1 beats/min (p > 0.3). The baseline fetal MAP of 54.3 ± 3.0 mm Hg increased nonsignificantly during the epinephrine infusion to 57.7 ± 3.2 mm Hg (p > 0.2), returning to baseline levels in the recovery period.

Arterial blood gas data are shown in Fig. 2. There were no statistically significant changes in pH, Po₂ or PCO2 during the infusion or recovery periods. The fetal arterial pH showed a nonsignificant decrease from a baseline value of 7.36 \pm 0.01 to 7.34 \pm 0.01 after 20 minutes of epinephrine infusion (p > 0.1) and remained at this slightly lower level for the remainder of the study.

There was a significant elevation in fetal mean plasma glucose concentration during the epinephrine infusion (Fig. 3). From a baseline value of $42.1 \pm 8.2 \text{ mg/dl}$ the plasma glucose level rose to 65.5 ± 5.9 mg/dl after 30 minutes of infusion (p < 0.05). At the completion of the recovery period the fetal glucose concentration, although not statistically elevated from the control, had not returned to baseline values, being 52.6 ± 11.7 mg/dl (p > 0.4). The fetal mean plasma insulin concentration demonstrated a delayed rise after cessation of the epinephrine infusion. Control plasma insulin levels of 9.6 \pm 2.5 μ IU/ml decreased to 4.5 \pm 1.8 $\mu IU/ml$ (p > 0.1) after 20 minutes of epinephrine infusion and then rapidly increased to 32.4 ± 18.0 µIU/ml 15 minutes after cessation of the infusion (p < 0.05).

Fetal blood lactate levels progressively increased throughout the experimental period, reaching significantly elevated levels in the recovery period (Fig. 4). Baseline lactate values of $36.1 \pm 4.8 \text{ mg/dl}$ increased to $48.7 \pm 4.5 \text{ mg/dl}$ (p = 0.06) after 40 minutes of epinephrine infusion and peaked at 54.1 ± 5.7 mg/dl (p < 0.05) 30 minutes after cessation of the infusion.

The average basal epinephrine concentration was $435.7 \pm 36.0 \text{ pg/ml}$. We did not observe a marked decline in basal epinephrine concentration with advancing gestational age. The individual calculated epinephrine clearance rate values for the fetus of the diabetic ewe are plotted against gestational age in Fig. 5. Plasma catecholamine samples from two fetuses were inadver-

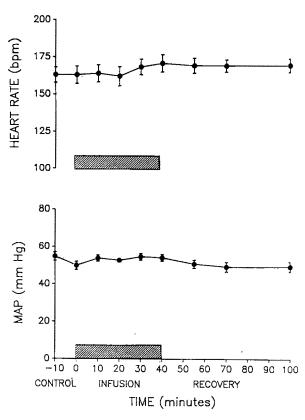


Fig. 1. FHR and MAP before, during, and after infusion of epinephrine 0.1 μ g/kg/min, n = 12. Data represent mean ± SEM.

tently lost during assay, and the data are available from 10 animals only. In the normal ovine fetus the epinephrine metabolic clearance increases as gestation advances.4 In the fetus of the diabetic ewe, however, there is no significant alteration in epinephrine clearance rate with increasing gestational age. In this species term is 150 ± 5 days' gestation. Because of difficulties in maintaining the preparation beyond 140 days' gestation, we were unable to obtain later studies.

Plasma epinephrine production rates were calculated by multiplication of the mean basal plasma epinephrine level by the individual metabolic clearance rate. Plasma epinephrine production rates remained constant relative to gestational age in the fetus of the diabetic ewe. The mean epinephrine production rate for study fetuses <135 days' gestation (n = 5) was 15.4 \pm 1.5 ng/min and did not differ from those of older fetuses (>135 days' gestation, n = 5) at 13.9 ± 2.0 ng/min (p > 0.5).

Comment

The catecholamine content of the ovine fetal adrenal gland increases progressively as gestational advances, plateauing at 130 to 140 days' gestation.2 The adrenal content of norepinephrine decreases, whereas that of epinephrine increases with gestation.1.2 The rise in epi-

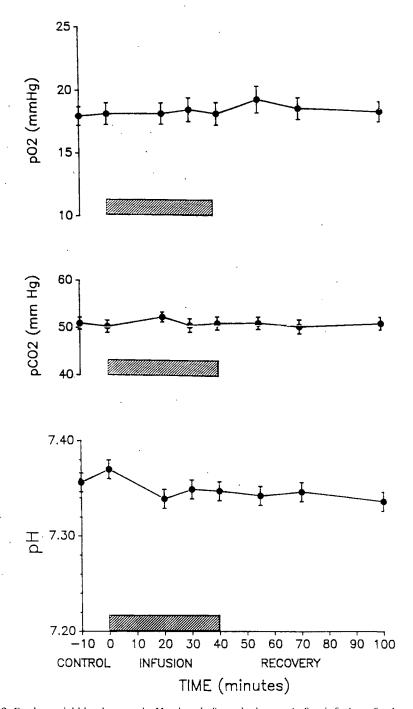
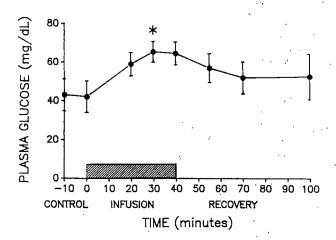


Fig. 2. Fetal arterial blood gas and pH values before, during, and after infusion of epinephrine 0.1 $\mu g/kg/min$, n=12. Data represent mean \pm SEM.

nephrine content is presumed to be a secondary effect of the increase in cortisol production in the adrenal cortex of the maturing fetus.'

With advancing gestation there is an increase in renal blood flow, glomerular filtration, and amniotic fluid concentration of catecholamines.^{8,9} This is suggestive of an increase in the renal clearance of catecholamines toward the end of gestation.

In the fetal lamb the plasma concentrations of catecholamines decrease with increasing gestational age³ although there is no alteration in catecholamine production rate.⁴ The decreased plasma catecholamine concentration coupled with the increase in amniotic fluid concentration implies increased clearance of these vasoactive agents with advancing gestation. It has been previously shown in the chronically catheterized nor-



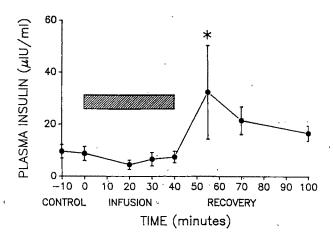


Fig. 3. Fetal plasma glucose and insulin levels before, during, and after infusion of epinephrine 0.1 μ g/kg/min, n = 12. Data represent mean \pm SEM. Asterisk, p < 0.05.

mal ovine fetus that the metabolic clearance rate of epinephrine is gestation dependent, with a rapid maturation in clearance after 134 days' gestation.⁴

The fetus of the diabetic woman with poor glucose control classically demonstrates delayed organ maturation, most typified by a retardation of pulmonary function. 10,11 Fetal lung maturation is influenced by several hormones, including cortisol, thyroxine, triiodothyronine, prolactin, and insulin.12-14 Parket et al.15 reported a reduction in the umbilical serum concentrations of cortisol in infants of women with diabetes in whom respiratory distress syndrome developed. Saltzman et al.16 noted a decrease in the umbilical cord prolactin concentration of infants of diabetic mothers. Cortisol acts as an inducer of fetal enzyme activity, and catechol-O-methyltransferase activity may potentially be impaired in the fetus of the diabetic mother, resulting in a reduction in clearance of released catecholamines. We hypothesized that as a marker of organ immaturity the metabolic clearance rate of epinephrine would be delayed in the fetus of the diabetic ewe.

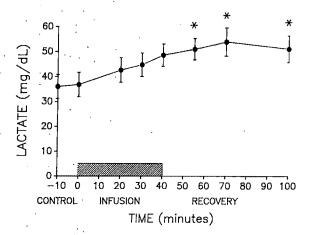


Fig. 4. Fetal blood lactate levels before, during, and after infusion of epinephrine $0.1 \,\mu\text{g/kg/min}$, n=12. Data represent mean \pm SEM. Asterisk, p < 0.05.

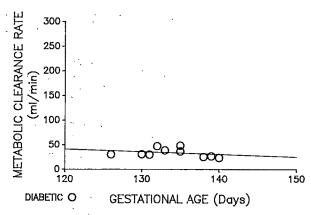


Fig. 5. Epinephrine metabolic clearance rate versus gestational age in fetus of diabetic ewe, n = 10. Data represent mean \pm SEM.

'An absence of maturation of epinephrine clearance in the fetus of the diabetic ewe throughout the gestations examined has been shown. Unfortunately, the animal model limited the number of animals evaluated at very early and late gestational ages. We speculate that the fetal epinephrine metabolic clearance rate increases later in gestation in the diabetic ewe, with the attainment of values equivalent to those of Palmer et al.4 and Padbury et al., 17 but were technically unable to continue our preparations beyond 140 days. Possible solutions to this question could be to perform clearance studies on the lambs immediately after birth, although this would entail separating the lamb from the ewe, thereby removing the lamb from its physiologic condition and invalidating the method. Another alternative is to administer thyroxine or corticosteroids in utero to the fetal lamb and repeat the metabolic clearance rate studies to assess if maturation can be induced. Cortisol is believed to activate the synthesis of epinephrine in the adrenal medulla by inducing the activity of the enzyme phenylethanolamine-N-methyltransferase. Because Parker et al. noted in humans an association between neonatal respiratory distress syndrome and the degree of maternal hyperglycemia, the clearance studies could be repeated with strict control of maternal hyperglycemia.

We attempted to assess the metabolic clearance rate of epinephrine in a basal physiologic state but were only partially successful. There were no significant alterations in FHR, MAP, or arterial pH; however, there were significant increases in plasma glucose and blood lactate levels with suppression of plasma insulin during epinephrine infusion, followed by rebound hyperinsulinemia. It is probable that there was significant peripheral vasoconstriction in spite of no significant alteration in measured fetal cardiovascular parameters. This would account for the rise in lactate. This effect has been observed in previous investigations with the constant infusion method.4, 17, 19 It would be worthwhile to conduct similar studies with lower epinephrine infusion concentrations to assess the physiologic thresholds for the metabolic effects of epinephrine in the ovine fetus.

We present the first examination of catecholamine clearance mechanisms in an ovine diabetic model. There appears to be a delay in the fetus of the diabetic ewe to clear epinephrine. This alteration may be a secondary effect of a generalized delay in organ maturation that has been observed in infants of mothers with uncontrolled hyperglycemia. We speculate that an inability to clear stress-released epinephrine may reduce the ability of such fetuses to tolerate stress.

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Effects of hyperglycemia on mitochondrial morphology in the region of the anterior neuropore in the explanted rat embryo model: Evidence for a modified Reid hypothesis as a mechanism for diabetic teratogenesis

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Congenital malformations are the leading cause of the increased perinatal mortality in the infants of insulin-dependent diabetic mothers. The mechanisms(s) of diabetic teratologic development has yet to be defined. Hyperglycemia is known to depress aerobic metabolism in many organisms and cell lines. Reid hypothesized that exposure to hyperglycemia could result in decreased mitochondrial biogenesis in embryonic cells. Should these cells suddenly be changed to an environment of lower glucose concentration, decreased energy capabilities would exist until sufficient numbers of mitochondria could be regenerated. Such cells may not be capable of meeting temporal-spatial requirements, thereby resulting in structural abnormalities. In our study the explanted rat embryo model demonstrated that in the head-fold region hyperglycemia produced morphologic alterations of mitochondria but no difference in the number of mitochondria per cell. Specifically, embryos cultured in euglycemia demonstrated orthodox mitochondrial configuration, whereas those cultured in hyperglycemia had mitochondria in a condensed configuration. These findings were reversible. A modification of Reid's original hypothesis may provide an explanation for the mechanism of diabetic teratologic development. (AM J OBSTET GYNECOL 1991;165:1661-6.)

Key words: Congenital malformations (diabetic), mitochondria (morphology), hyperglycemia

Congenital malformations remain the major factor in the increased perinatal mortality experienced by the infants of insulin-dependent diabetic mothers. These malformations affect numerous organ systems and have a wide range of expression. It is therefore argued that the teratogenic agent acts early in gestation at the cellular level in a universal manner. Because the exact teratogenic mechanism remains undefined, hyperglycemia continues to be implicated in the teratogenic process of this common maternal metabolic disorder. 5-5

One of the problems in elucidating the mechanism(s) resulting in the malformations is the myriad of metabolic, hormonal, and substrate alterations evidenced in insulin-dependent diabetics. The explanted rat embryo model allows the investigator to accurately control and

manipulate the environment of the developing embryo.⁶ The head-fold region of the developing rat embryo has been demonstrated to be especially sensitive to environmental alterations. Failure of closure of the anterior neuropore has served as a morphologic marker for teratogenic effects.^{7, 8}

Extreme fluctuations in maternal glucose levels are not uncommon in pregnancies complicated by insulindependent diabetes. On the basis of metabolic studies in yeast and some cell cultures, Reid9 hypothesized that sudden swings in glucose levels could be more teratogenic than constant hyperglycemia. Specifically, cells exposed to hyperglycemia are known to switch to anaerobic metabolism. This alteration in metabolic activity is described as the Crabtree effect and is well documented in the literature. 10-13 Reid hypothesized that this metabolic switch resulted in a down-regulation in mitochondrial biogenesis, as evidenced in studies in crabtree-positive yeast. 10-13 Should a cell exposed to hyperglycemia and functioning in an anaerobic fashion be suddenly changed to a euglycemic environment, Reid went on to hypothesize, there would be a time interval before adequate mitochondrial numbers had been regenerated so as to allow for adequate energy production to meet cellular demands. This time interval was designated as the energy gap. Should such an energy gap exists in an embryonic cell with a set program for development, the cell might not be able to meet its

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spatiotemporal requirements, with the result being a fetal anomaly, e.g., failure of proper closure of the anterior neuropore with resultant anencephaly. This specific lesion has been demonstrated to be 10 times more common in infants of diabetic mothers¹⁴ than in infants of nondiabetic mothers. Anterior neuropore defects, including failure of closure and abnormal closure, are a common malformation in rat embryos cultured in hyperglycemic serum and have served as a morphologic marker of teratogenesis.^{7,8}

This study was designed to determine if hyperglycemia would (1) result in decreased mitochondrial numbers or (2) modify mitochondrial morphologic development.

Material and methods

The explanted rat embryo model as developed by New⁶ and further described by Sadler⁸ serves as the basis of the present study. This model and all procedures and handling were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center.

Sprague-Dawley rats were maintained on a 12-hour-light/12-hour-dark cycle and provided with food and water at will. Vaginal smears were examined for sperm the morning after overnight mating. When intravaginal sperm was found, day 0 of the pregnancy was defined as the preceding midnight. Pregnant females were housed separately with food at will in 12-hour-light/12-hour-dark cycles.

Serum for culture media was prepared by cardiac puncture and exsanguination of Sprague-Dawley retired male breeder rats that were under halothane anesthesia. The blood was immediately centrifuged, and the serum was separated and immediately heat inactivated at 56° C for 30 minutes. Euglycemic serum was thus defined with a normal serum glucose level of approximately 125 mg/dl. Hyperglycemic serum was prepared by adding an additional 2.5 mg of D-glucose to each milliliter of the previously prepared euglycemic serum, for a final glucose concentration of approximately 400 mg/dl. Before the embryos were explanted, the serum was pregassed with 95% oxygen and 5% carbon dioxide and warmed to 38° C. Paired hyperglycemic and euglycemic serum was defined as that prepared from the same retired male breeder.

Pregnant females at days 10 to 11 were placed under halothane anesthesia, a laparotomy was performed, and the pregnant uterine horns were extirpated and transferred to a sterile culture dish containing physiologic saline solution at 38° C. Adjacent embryos were dissected free by opening the anterior wall of the uterus under direct vision with a dissecting microscope. Reichert's membrane, with the attached trophoblast and parietal ectoderm, was removed and opened. The visceral

yolk sac, amnion, and ectoplacental cone were left intact and explanted with the embryo. The paired embryos were then transferred to paired hyperglycemic and euglycemic sera. Each embryo was cultured in 3 ml of serum in a pregassed, closed flask (volume 10 ml) for 4 hours in an incubator at 38° C. At the end of 4 hours, the serum was replaced with pregassed, paired hyperglycemic and euglycemia sera (as previously determined) and the culture continued at 38° C for an additional 4 hours. Four embryos were explanted from one dam (dam F) with one embryo cultured in hyperglycemic sera for 8 hours and one in euglycemia sera for 8 hours as described. The other two embryos were cultured in alternating sera with one embryo cultured in hyperglycemic serum for 4 hours, then in euglycemia serum for the final 4 hours, and the other embryo cultured in euglycemic serum for 4 hours, followed by hyperglycemic serum the final 4 hours.

At the conclusion of the 8-hour culture interval, the paired embryos were viewed under the dissecting microscope. Only those pairs for which adequate embryonic heart activity was demonstrated (heart rate >100 beats/min) were prepared for cellular studies.

Crown-rump lengths were obtained at the end of the 8-hour incubation. Embryos were then immediately fixed in Karnovsky's fixative for 4 hours, followed by 2% glutaraldehyde for 48 hours. Embryos were then placed in 1% osmic acid for 1 hour. Serial alcohol dehydration was performed and the embryos then embedded in resin (L.R. White, Ted Pella, Inc.). Thick sections were stained with toluidine blue and blocks trimmed for electron microscopic studies. Cells of the anterior neuropore were examined with the transmission electron microscope. For each embryo of the paired embryo cultures, serial sections in the region of the anterior neuropore were performed. Transmission electron micrographs were obtained at magnifications of ×8120 to ×28,000.

Mitochondria were classified as to morphologic type (type I or type II) in accordance with described criteria on the basis of shape, matrix density, and cristae width. ¹⁵ Specifically, type I mitochondria have an elongated longitudinal cross section, a dark matrix density, and an average cristae width of 80 nm. Type II mitochondria are circular in cross section, have a light matrix, and have a mean cristae width of 45 nm. The types of ≥60 mitochondria in the region of the anterior neuropore were identified for each embryo (range 60 to 164).

The transmission electron micrographs were transferred to a MacIntosh computer (Apple Computers) by means of a Hewlett-Packard Laserscanner and graphic software. Cytoplasmic, nuclear, and mitochondrial areas were determined by graphic software for a minimum of 7 cells in the region of the anterior neu-

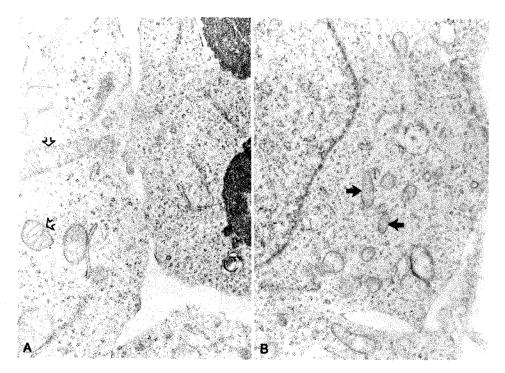


Fig. 1. Transmission electron micrographs of embryonic cells in region of anterior neuropore of paired rat embryos explanted at 10 days and cultured for 8 hours. A, Euglycemic serum as culture media for 8 hours with open arrow demonstrating typical type II mitochondrial morphologic characteristics. B, Hyperglycemic serum as culture media for 8 hours with closed arrow demonstrating typical type I mitochondrial morphologic characteristics. (Original magnification ×28,000.)

ropore for each embryo (range 7 to 12 cells). The number of mitochondria observed in each cell was recorded. The average mitochondrial area, the average number of mitochondria per cytoplasmic area, and the ratio of the mitochondrial area to cytoplasmic area for each embryo were then determined. During the computer analysis, the examiner was blinded as to whether the embryo had been cultured in euglycemic, hyperglycemic, or alternating sera.

Statistical studies were performed for the paired embryos cultured in the euglycemic and hyperglycemic serum. Nonparametric statistical analyses were used for the comparison of the morphologic types of mitochondria (percentages). The number of mitochondria per cytoplasmic area, average mitochondrial area, and mitochondrial area/cytoplasmic area ratios were compared by the paired t test. Statistical significance was defined as p < 0.05.

Results

Successful cultures were completed in four of five paired studies with two embryos from each of dams A through E and in four of four embryos cultured from dam F. For one pair of embryos no cardiac motion was noted for the embryo cultured in hyperglycemic sera. Therefore the overall rate of culture success was 92%

(11 of 12 explanted embryos). No morphologic anomalies were visualized at the conclusion of the 8-hour culture interval.

A significant difference in embryonic growth was demonstrated for paired embryos cultured in hyperglycemic sera versus euglycemic sera, with an average decrease in final crown-rump length of 10.5%. Paired t test demonstrated a significance of p < 0.02 for the final crown-rump lengths of the paired embryos.

Fig. 1 demonstrates comparison of mitochondria typically seen in embryos cultured in euglycemic and hyperglycemic serum. No difference in the number of mitochondrial per cytoplasmic area (mitochondrial density) was demonstrated between those embryos grown in euglycemic sera and the paired embryos cultured in hyperglycemic sera (Table I, p = 0.59).

The average mitochondrial area (paired t test, p = 0.004) and the ratio of mitochondrial area to cytoplasmic area (paired t test, p = 0.04) were determined to be decreased for those embryos cultured in hyperglycemic sera as compared with the paired embryos cultured in euglycemic sera (Table I).

The percentage of mitochondria in the condensed form (type I) as compared with the orthodox configuration (type II) was increased for those embryos cultured in hyperglycemic sera versus the paired embryos

Table I. Comparison of mitochondrial parameters and crown-rump lengths for paired embryos cultured in hyperglycemic and euglycemic media

	Type of culture media			
	Hyperglycemic	Euglycemic	Statistics	
Crown-rump length (mm)	3.48 ± 1.06	3.88 ± 1.1	p < 0.02 (paired t test)	
Mitochondria per cytoplasmic area (No./ μm^2)	0.042 ± 0.230	0.036 ± 0.018	p = 0.59 (paired t test)	
Average mitochondrial area (µm²)	0.023 ± 0.011	0.051 ± 0.021	p = 0.004 (paired t test)	
Mitochondrial/cytoplasmic area ratio	0.038 ± 0.013	0.072 ± 0.014	p = 0.04 (paired t test)	
Mitochondria in type I configuration (%)	78.8 ± 12.6	20.4 ± 6.9	p = 0.03 (Wilcoxon signed-rank)	

Table II. Effects of alteration of glucose environment on mitochondrial morphologic for embryos of dam F

	Constant euglycemia	Constant hyperglycemia	Hyperglycemic for 4 hr then euglycemic for 4 hr	Euglycemic for 4 hr then hyperglycemic for 4 hr
Crown-rump length (mm) Mitochondria per cytoplasmic area (No./μm²) Average mitochondrial area (μm²) Mitochondria/cytoplasmic area ratio Type I mitochondria (%)	4.0	3.4	3.2	3.6
	0.032	0.014	0.023	0.018
	0.063	0.028	0.080	0.011
	0.09	0.02	0.08	0.01
	30	64	17	67

cultured in euglycemic sera (Table I, Wilcoxon signed-rank test, p < 0.02).

Reversed cultures in the embryos cultured from dam F demonstrate the mitochondrial studies to be dependent on the type of sera used in the final 4 hours of the culture. Mitochondrial morphologic type and dimensions reversed within 4 hours, as demonstrated in Table II. In addition, the final crown-rump lengths were decreased for those embryos exposed to hyperglycemia, with the most significant decrease experienced by the embryo initially cultured in hyperglycemia then suddenly changed to euglycemic media (Table II).

Comment

Congenital malformations are the major cause of the increased perinatal mortality experienced by the offspring of insulin-dependent diabetic mothers. Hyperglycemia remains the factor most often cited as being involved in the teratogenic process; however, the exact mechanism has yet to be elucidated. The Crabtree effect (reversed Pasteur effect) occurs when a cell exposed to hyperglycemia experiences a decrease in aerobic metabolism and a switch to the glycolytic pathways. Reid⁹ hypothesized that a cell so exposed would experience a decrease in mitochondrial biogenesis. Should this cell suddenly be removed to an environment with a lower glucose concentration, the cell would experience a time interval during which energy production capability would be reduced until such time as

appropriate mitochondrial numbers could be regenerated. This time interval Reid defined as the *energy gap*. For a developing embryo, such a gap could be expected to result in cells incapable of meeting temporal and spatial requirements, thereby producing structural defects. To date, this hypothesis has not been studied in mammalian embryos.

Our data do not, however, support the decrease in number of mitochondria in rat embryos exposed to hyperglycemia, as would be expected by Reid's original hypothesis. Rather, no statistical difference in the number of mitochondria per cytoplasmic area (mitochondrial density) existed in those paired embryos cultured in hyperglycemic versus euglycemic sera. The data do, however, demonstrate a marked difference in mitochondrial morphologic features as evidenced by mitochondrial size and morphologic subtype.

Alterations in mitochondrial morphologic subtype have been clearly demonstrated to indicate alterations in the metabolic activity of the mitochondria. Type II mitochondria are indicative of an energized cell and are induced with the addition of intracellular adenosine triphosphate. Predominantly type I mitochondrial configurational changes are induced by the addition of intracellular adenosine diphosphate and are thus indicative of a lower energy state. Indeed, the appearance of type I mitochondria requires a phosphate acceptor, e.g., adenosine diphosphate, in addition to a substrate. Our data thus provide evidence for alteration

in cellular metabolic activities in cells of the developing rat embryo exposed to hyperglycemia, an alteration that is further demonstrated to be reversible in matched embryos.

Hyperglycemia certainly could result in alterations of mitochondrial morphologic features by other mechanisms or other messengers. For example, recent studies by Horton and Sadler¹⁹ described an alteration in mitochondrial morphologic type of explanted rate embryos exposed to β-hydroxybutyrate. Specifically, a decrease in type I mitochondria was demonstrated in those cells exposed to this ketone. Ketones are metabolized within the mitochondria; therefore it is speculated that this substrate may serve to activate the mitochondria and increase metabolic activity.20

Sadler and Hunter²¹ and Akazawa et al.²² have demonstrated that exposure to hypoglycemia also can be teratogenic. The latter study demonstrates that sudden alterations in glucose concentrations can have profound effects on the teratogenic process. For those explanted embryos cultured in euglycemia sera for 48 hours, the incidence of malformations was 0%. Embryos were then cultured in euglycemic sera for 8 hours, exposed to hypoglycemic sera for 1 hour, and then cultured in euglycemic sera for the final 39 hours. The incidence of major malformations was 7.1%. When the experiment was performed with 33.3 mmol/L glucose sera rather than euglycemia media, the incidences of major malformations was 0% and 21.2%, respectively, for those embryos not exposed and exposed to brief hypoglycemia. These results were abolished when glucose was added to the hypoglycemic sera. Furthermore, the addition of insulin to the nonhypoglycemic sera did not produce major malformations.

These findings by Akazawa et al.22 are of major significance in that they demonstrate that sudden substrate changes are more teratogenic than constant exposure to hyperglycemia. That a significant incidence of malformations could be induced at a glucose level previously found not to be teratogenic in a constant culture environment argues against hypotheses centering on elevated metabolic byproducts of glucose (e.g., sorbitol) as the primary process of diabetic teratologic development. However, altered cellular energy production capabilities could provide such an explanation.

Another possible mechanism is altered phospholipid metabolism and biogenesis, such as the hypothesis described by Naftolin et al.28 for the developing embryo and yolk sac. They speculate that alteration of the yolk sac membrane results in embryonic asphyxia. Abnormalities in the yolk sac membrane also are supported by Sussman and Matschinsky.24 Arachidonic acid25 and myoinositol²⁶ supplements are known to abolish the teratogenic effects of hyperglycemia. Because mitochondria are the site of conversion of pyruvate to acetyl coenzyme A (the major fatty acid precursor) and the site of fatty acid metabolism, altered mitochondrial function would be expected to result in altered lipid metabolism.

Further evidence for the potentially increased teratogenesis of sudden alterations in glucose levels is provided by the decreased crown-rump lengths for embryo from dam F, which was cultured in hyperglycemia followed by euglycemia. The strong correlation between growth abnormalities and malformations for embryos subjected to a diabetic environment has been well established both in vivo^{7, 8, 25} and in vitro.²⁷ The most profound decrease in embryonic growth was for the embryo initially cultured in hyperglycemia then suddenly switched to a euglycemic environment. This is as predicted should sudden alterations in glucose milieu be a mechanism of diabetic teratogenesis.

We therefore suggest that the Reid hypothesis be modified to describe a metabolic gap to result from a modification of the mitochondrial metabolic capacity (as evidenced by mitochondrial morphologic characteristics) rather than a decrease in the number of mitochondria or mitochondrial density. A swing from a hyperglycemic environment to a euglycemic environment would still result in either (1) a decreased energy production capacity adequate to meet spatiotemporal demands or (2) decreased phospholipid production with profound effects on cellular membranes, cellular communication, and calcium channels of the developing embryo. This effect(s) would continue until such time as the mitochondria had altered to a more active or appropriate metabolic morphology. The demonstration of the reversibility of mitochondrial morphology adds further support for this hypothesis.

Current studies continue to demonstrate the effect of episodic hyperglycemia on embryonic growth and development as compared with constant hyperglycemia. Further studies of precise biochemical markers for intracellular metabolic pathways, including fatty acid metabolism and glucose metabolism, are in progress.

The potential for embryonic damage by sudden metabolic swings has been suggested by several investigators.21, 23, 24 Demonstration of support for the modified Reid hypothesis would have important implications in the care and management of the insulin-dependent diabetic woman in the first trimester of pregnancy, i.e., insulin schedules and dosages structured to avoid rapid altertaions of levels of maternal glucose.

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Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to disease, hypertension these and other risks.

disease, hypertension, tetal adnormalines. Practitioners should be ramiliar with the available information relating to these and other risks.

1. Thromboembolic disorders and other vascular problems: increased risk of thromboembolic and thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of Idiopathic deep vein thromboels and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonsers. Overall excess mortality from PE or stroke was 1.3-3.4 detaths/year/100,000 users and increased with age referbrovascular disorders: In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stoke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Itakers is evident, but the numbers are too small to lipsify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (Mi) and hemorrhagic stroke. Excess mortality was associated with age and smoking, A. 1983 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (Mi) and hemorrhagic stroke. Excess mortality was associated with smoking, age, and Mi were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nontheumatic heart disease was greater in ever-users. In the Walant Creek Study, subarachnolib hemorrhage risk was associated

Estimat	ed Mortality Rate	from Myc	ocardial Infarction	per 100,000 Women	Year		
-	Aged 30-39:	Users	Nonusers	Aged 40-44:	Users	Nonusers	
All smokers		10.2	2.6		62.0	15.9	
≥15 cigarettes daily		13,0	5.1		78.7	31.3	
<15 cigarettes daily		4.7	0.9		28.6	5.7	
Nonsmokers		1.8	1.2		10.7	7.4	
Smokers and nonsmokers		5.4	1.9		32.8	11.7	

Nonsmokers

1.8
1.2
3.2.8
1.7

**Nisk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose not between entityel estratiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estragen doses were not longer prescribed. Studies on TE risk with progestogen-only OCs have not ben done. Cases have been reported for thase; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored. OCs may be divided into those with <000 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk, for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Later group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myccardial ischemia, occurs with high progestational activity Consider amount of both steroids in choosing OC.

Risk is: A-very high; B-high; C-moderate; D-low.	Age:	<30	30-39	≥40
Heavy smokers (≥ 15/day)		C	В	A
Light smokers (<15/day)		D	С	В
Nonsmokers, no predispositions		D	C,D	С
Nonsmokers, with predispositions		C	C.B	B.A

Re alert to earliest manifestations of TE disorders (eg. thrombophlebilis, PE, cerebroascular insufficiency, comparay artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, welgn isks of postsurgery TE complications against contraceptive needs. RCOP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis, 2. Deutar lesions; Neuro-ocular lesions such as optic neurities or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete foss of vision; proptosis or diptopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. 3. Carcinoma: Long-term estrogen in certain animals increases carcinomas and replasms, such as those of breast, ulerus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benipn and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial carcinomic incidence since 1968 in areas with cancer-reportions systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred

nearly always with no-longer-marketed spauential OCs. No statistical association is reported suggesting increased enformet cancer with combination or progestiogen-enity OCs. Studies show no increased breast cancer with OCs or estrogens. Howe, greater risk was noted in OC users with beingin breast disease or long-term (2-4 years) use. One study found greater risk reast cancer with 2-4 years OCs or grint to list full-time programs, Pisk to breast cancer in connection with other reactors and in virtorio subjourps. Decrease in breign breast tumors in OC users is well known. Some studies suggest increased risk of cervical dysptasis, GN, encoion, and carcinoma in long-term users. Cervical microgranical hypotyplasis of the programs of the studies of increased cancer risk with OCs or extension and the studies of increased cancer risk with OCS or extension and the studies of increased cancer risk with OCS or extension and the studies of increased cancer risk with occare and take disponetic measures to rule out malignancy. Carchityl monitor users with strong family bistory of the causes and take disponetic measures to rule out malignancy. Carchityl monitor users with strong family bistory of the causes and take disponetic measures to rule out malignancy. Carchityl monitor users with strong family increased or with programs of cervical disposite 4. Hepatic adenomase, etc. have been associated with OCs, in 1 study OC formulations with high "hormunal potency" we associated with higher risk, as was an include the programmy of OC use when submitting specimens. Endocrine and liver function tests may be attered; repeat abnormal tests after stopp for 2 months. These have been observed: increased 65P retention and other abnormalities in liver function tests; increase prothorobin and coaguistion factors VII, VII, IX, X; decreased antiformobin ill, increased platelet against prothorobin and coaguistion factors VII, VIII, IX, X; decreased antiformobin ill, increased platelet against particular thyroid-binding globulin leading to increased circulating total thyroid homone (PBI, T*); decreased T* gutake, unaltered f* T*, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceri phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of protonged OC in on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs my mask onset climacteric. Cervical chiamydial and gonorrheal prevalence is increased; do not assume OC protects against chiamydial P HIV sempositivity was associated with OC use. Information for patients. See patient liabeling. Oring interactions. OC effectiven may be decreased and more BTB occur with inampin; soniazid, ampicillin, neomycin, periolicini, eticinic, introdurantion, griseotulvin, babiliurates, phenytoin, carbamazepine, primidone, phenyibutazone, analgesi tranquilitzers, antimigraines. OCs may after effectiveness of oral anticonvulsants, tranquilitzers, tricy antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminoph Adverse reactions: increased risk of the following senious adverse reactions as been associated with Occurrence and thrombosis; curenbal thrombosis; cerebral thrombosis, seriou-ocular fedicions, with or without intra-abdomin

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Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women

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To assess the longitudinal changes in insulin release and insulin sensitivity in nonobese normal women during gestation, six women were evaluated with oral glucose tolerance testing, body composition analysis, intravenous glucose tolerance tests, and the hyperinsulinemic-euglycemic clamp before conception, at 12 to 14 weeks, and at 34 to 36 weeks' gestation. There was a significant increase in the insulin/glucose ratio (p=0.028) during the oral glucose tolerance test during gestation. There was also a significant 3.0- to 3.5-fold increase throughout gestation in first-phase (p=0.001) and second-phase (p=0.0001) insulin release during the intravenous glucose tolerance test. Peripheral insulin sensitivity was estimated as the glucose infusion rate (in milligrams per kilogram fat-free mass per minute) during the hyperinsulinemic-euglycemic clamp. There was a significant (p=0.0003) 56% decrease in insulin sensitivity through 36 weeks' gestation. These results are the first to prospectively evaluate the longitudinal changes in maternal carbohydrate metabolism from the time before conception through late gestation with newer methods such as the hyperinsulinemic-euglycemic clamp. (Am J Obstet Gynecol 1991;165: 1667-72.)

Key words: Carbohydrate metabolism, insulin release, insulin sensitivity, pregnancy

Pregnancy has often been characterized as having a diabetogenic effect on normal carbohydrate metabolism, as manifested by hyperglycemia and hyperinsulinemia in response to maternal feeding. This combination of elevation in postprandial glucose and insulin response during gestation has been cited as the basis for decreased peripheral insulin sensitivity during pregnancy. The methods by which insulin sensitivity has been quantified during gestation have usually been equated with insulin response to a glucose challenge² or the rate of glucose disappearance during an intravenous glucose tolerance test (GTT). However, neither of these methods may adequately estimate peripheral insulin sensitivity.

Furthermore, the mechanism by which the anabolic changes in early pregnancy, such as increased maternal fat deposition, are mediated remain controversial. Various reports have described either increased,⁴ decreased,⁵ or unchanged⁶ insulin sensitivity or insulin

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release⁷ in early pregnancy. These studies, however, relied on either postpartum evaluation⁷ or cross-sectional studies⁴ to estimate the changes in carbohydrate metabolism in early pregnancy.

The purpose of this prospective, longitudinal study

The purpose of this prospective, longitudinal study was to characterize the normative changes in insulin response and insulin sensitivity in healthy nonobese pregnant women. We chose to evaluate nonobese women as a distinct group from obese women, because of the potential affect of obesity on carbohydrate metabolism during gestation.⁸

Methods

Subjects. This study was performed in the General-Clinical Research Center at the Medical Center Hospital of Vermont from 1985 through 1989. The study was approved by the hospital's institutional review board, and informed consent was obtained from each subject before the study. Six healthy white women were recruited to participate. All had at least one previous uncomplicated term pregnancy. None had a family history of diabetes mellitus in a first- or second-degree relative or abnormal glucose tolerance in a previous pregnancy. None of the subjects were breast-feeding or using oral contraceptives, other medications, or tobacco. All were planning to conceive as soon as the baseline pregravid studies were completed.

Experimental protocol. The study design consisted of a series of experiments initiated before conception (five of six subjects during the follicular phase of the menstrual cycle) and repeated again during early (12)

Table I. Pregravid morphologic data

Age (yr)	31.8 ± 5.5*
Height (cm)	166.9 ± 6.9
Weight (kg)	56.5 ± 8.4
Body mass index (wt/ht²)	20.3 ± 2.3
Body fat (%)	17.8 ± 5.6
Time between pregravid studies and con-	2.2 ± 1.3
ception (mo)	

^{*}Mean ± SD.

to 14 weeks) and late (34 to 36 weeks) gestation. Each subject was instructed in a standard diet by the Clinical Research Center nutritionist 2 weeks before each study. The diet was designed to standardize nutritional intake for all subjects, maintain weight before conception, and apropriately increase weight during gestation.

All subjects were admitted to the General Clinical Research Center for a 3-day period of study. These studies were sequentially performed during the 3-day protocol: day 1, oral GTT; day 2, body composition analysis and intravenous GTT; day 3, the hyperinsulinemic-euglycemic clamp.

Oral GTT. Before conception all subjects were given a 75 gm oral GTT, as defined by the National Diabetes Data Group,⁹ and during pregnancy all subjects were given a 100 gm oral GTT. Normal glucose tolerance during gestation was defined by the criteria of Carpenter and Coustan.¹⁰ The changes in oral glucose tolerance over time were analyzed as the total area under the curve and the individual time points (fasting, and 1, 2, and 3 hours). The plasma glucose concentrations were determined by the glucose oxidase method with a Yellow Springs glucose analyzer. Insulin concentrations were measured by radioimmunoassay by the method of Starr and Rubenstein.¹¹

Intravenous GTT. For the intravenous GTT, we infused 0.5 gm/kg of glucose as a bolus over 3 minutes, and samples of glucose and insulin were obtained at 0, 1, 3, 5, 10, 15, 30, 45, and 60 minutes after the start of the infusion. The K value, or the rate of glucose disappearance from the circulation, was estimated according to the method of O'Sullivan et al. 12 The first-phase insulin response was estimated by measuring the area under the glucose curve from 0 to 5 minutes and the second phase, by measuring the area under the curve from 5 to 60 minutes.

Body composition analysis. Each subject's fat-free mass and body fat were estimated by underwater weighing with simultaneous correction for residual volume by helium dilution.¹⁸

Hyperinsulinemic-euglycemic clamp. The hyperinsulinemic-euglycemic clamp was performed as described by DeFronzo et al.¹⁴ to estimate insulin sensitivity. The hyperinsulinemic-euglycemic clamp involves a primed constant infusion of insulin, 40 mU/m² to

achieve a high level of insulin (approximately 100 $\mu U/ml).$ A variable infusion of 20% glucose was adjusted, on the basis of plasma glucose samples drawn every 5 minutes, to maintain plasma glucose at 90 mg/dl. The rate of glucose infusion during the last 40 minutes of a 2-hour infusion was then taken as the estimate of peripheral insulin sensitivity.

Statistical analysis. The data were expressed as the mean ± SD. Statistical analysis was performed by analysis of variance with repeated measures. The changes in early and late pregnancy were analyzed with a Dunnett test. Statistical analysis was performed with an SAS statistical package. Probability levels ≤0.05 were considered significant.

Results

The pregravid age, height, weight, body mass index, percent body fat and time between pregravid study and conception are shown in Table I. We defined nonobese subjects as those women having a pregravid percent body fat <25%.

Oral glucose tolerance. All subjects had normal oral glucose tolerance both before conception and during gestation. Since we used a greater (100 gm) oral glucose load during pregnancy than during the time before conception (75 gm), we estimated the changes in oral GTT results over time with the insulin/glucose ratio (Fig. 1). There was a significant increase (p = 0.028) in the area under the insulin/glucose ratio curve during gestation. The increase was significant in early (p = 0.04) but not late gestation. When the data were analyzed at each time point, there was a significant increase in the fasting (p = 0.03), 2-hour (p = 0.03), and 3-hour (p = 0.04) plasma insulin/glucose ratios during the course of study. These changes were significant for the fasting insulin/glucose ratio from early to late gestation (p = 0.05) and for the 2-hour insulin/glucose ratio from the time before conception through early gestation (p = 0.05).

Intravenous GTT. There was no significant change (p=0.72) in the rate of glucose disappearance or K value during pregnancy: pregravid, 1.84 ± 0.79 ; early pregnancy, 1.94 ± 0.62 ; and late pregnancy, 1.68 ± 0.39 . There was, however, a significant increase in the first- (p=0.001) and second-phase (p<0.0001) insulin response during the intravenous GTT (Figs. 2 and 3). These increases in first-phase insulin response were significant in both early (p=0.025) and late (p=0.025) gestation. The increase in second-phase insulin response was significant only in late gestation (p=0.001). The mean increases in insulin response by late pregnancy were 3.5-fold for first-phase insulin response and 3.0-fold for second-phase response, as compared with pregravid insulin responses.

Hyperinsulinemic-euglycemic clamp. There was a

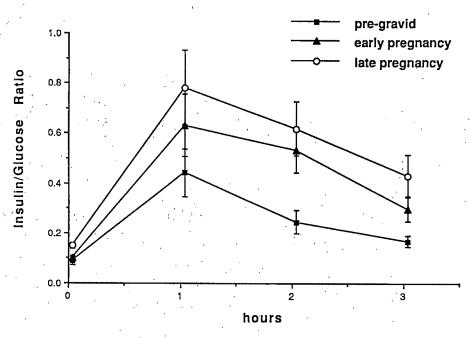


Fig. 1. Longitudinal changes in plasma insulin-glucose ratio during oral GTT (mean ± SD).

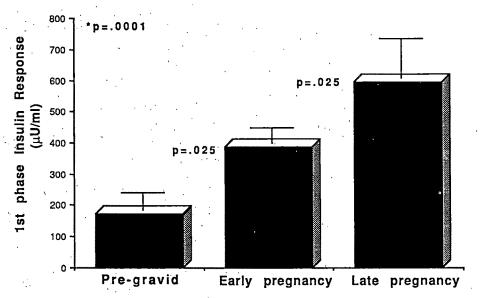


Fig. 2. Changes in first-phase insulin response during intravenous GTT (mean ± SD).

significant decrease (p = 0.0003) in the pregravid (10.5 ± 2.7) , early pregnancy (8.2 ± 2.5) , and late pregnancy (4.6 ± 1.3) glucose infusion rate (in milligrams per kilogram fat-free mass per minute), which represents a 56% decrease in insulin sensitivity from the time before conception through 34 to 36 weeks' gestation (Fig. 4). Thirty-nine percent of this decrease in insulin sensitivity occurred by 12 to 14 weeks' gestation (p = 0.04) and 61% of the decrease (p = 0.005) in insulin sensitivity by 34 to 36 weeks' gestation. These relationships remained essentially the same (pregravid, 8.7 ± 2.5 ; early pregnancy, 6.5 ± 2.0 ; and late preg-

nancy, 3.5 ± 1.0 ; p = 0.0001) when the glucose infusion rate was not corrected for fat-free mass in milligrams per kilogram per minute). Furthermore, there were no significant differences (p = 0.22) in the pregravid (97.6 \pm 18.7 μ U/ml), early (80.7 \pm 14.4 μ U/ml), or late (99.9 ± 27 μ U/ml) insulin concentration during the hyperinsulinemic-euglycemic clamp.

Comment

The findings are consistent with previous reports showing a progressive increase in insulin response to glucose during late gestation. Spellacy and Goetz² and December 1991 Am J Obstet Gynecol

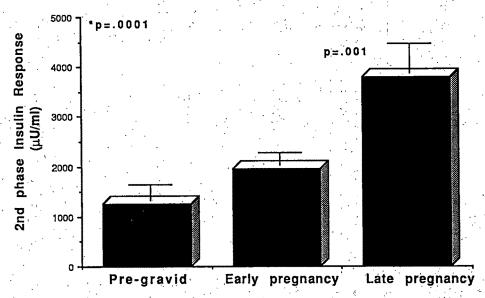


Fig. 3. Changes in second-phase insulin response during intravenous GTT (mean \pm SD).

Spellacy et al.15 showed a significant increase in insulin response to an intravenous GTT in mid to late pregnancy, when compared with the same subjects examined post partum. There was no significant difference, however, in insulin response at 13 to 15 weeks' gestation with the same study protocol. In contrast, our study examined baseline measurements before a planned pregnancy and showed a 120% increase in first-phase insulin response and a 50% increase in second-phase insulin response by 12 to 14 weeks gestation. The increases in late gestation were similar to those reported by Spellacy and Goetz.2 The reasons for the differences in results in early pregnancy remain unknown. We speculate that postpartum measures of insulin response may still be elevated postpartum in comparison with those obtained before conception.

The results of our study generally agree with previous reports showing a significant decrease in insulin sensitivity in late gestation. The studies of Fisher et al., 16 using a high-dose glucose infusion test, showed that normal pregnant women <85th percentile standard of body weight at 38 to 40 weeks' gestation had a decrease of about 80% in the insulin sensitivity index of that observed in a nonpregnant group. Buchanan et al.,17 with the minimal model technique, found that insulin sensitivity in normal pregnant women at 29 to 36 weeks' gestation was only one third of that of a group of normal nonpregnant women of a similar age and relative weight. Both these studies, however, used a crosssectional study design. Ryan et al.,18 as part of a study using the euglycemic clamp technique at 40 mU/m2/min, evaluated insulin sensitivity in nonpregnant women in comparison with a group of normal pregnant women, two of whom had repeat studies post

partum. They found that the pregnant women, in comparison with nonpregnant women, had glucose infusion rates that were decreased by 33% in late pregnancy. In the two pregnant women who were restudied 3 days postpartum, the glucose infusion rates returned to levels seen in the nonpregnant group. Of note, these studies were completed between 33 to 39 weeks' gestation, and the pregnant subjects' weight were a mean of 117% ideal body weight adjusted for pregnancy.

Our study differs from previous reports by the nature of the longitudinal study design and the method used to estimate insulin sensitivity. These experiments in healthy nonobese women on a standard diet allowed us to measure the changes in insulin sensitivity with minimal interference from factors other than pregnancy. The 56% decrease in insulin sensitivity by 34 to 36 weeks' gestation in normal pregnant women is comparable to the 66% decrease reported by Buchanan et al.17 and greater than the 33% reported by Ryan et al.18 but less than the 78% reported by Fisher et al.16 Whether these significant alterations in maternal metabolism during gestation have any permanent effects in these women remains to be examined. Our study did not address this question. We used only parous subjects in our protocol and did not evaluate these women post partum. A study using similar methods in nulligravid subjects before, during, and after their first pregnancy would be useful in answering this important question. For example, the available literature is contradictory regarding the importance of parity as a risk factor in the development of type II diabetes mellitus. 19, 20

All these estimates of insulin sensitivity in late pregnancy, however, are probably overestimates of true ma-

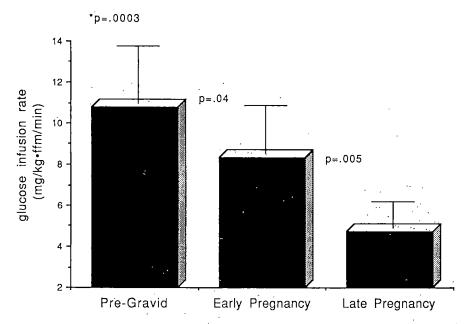


Fig. 4. Changes in glucose infusion rate during hyperinsulinemic-euglycemic clamp (mean ± SD).

ternal insulin sensitivity, because of the noninsulin-mediated glucose transport to the fetus by the placenta. Because of the limitations of our method, we were unable to distinguish the amount of the exogenous glucose infusion taken up by maternal tissues from that being transported to the fetus. Estimates of the partition of maternal glucose production between the conceptus and maternal tissues, however, has been examined by Hay et al.21 with the late-gestation pregnant ewe model. Approximately one third of maternal glucose utilization was accounted for by uterine glucose uptake (uterus, placenta, and fetus). Additional studies using this model by the same investigators have shown that fetal glucose use had a linear correlation (r = 0.89) with maternal arterial plasma glucose concentration.22 However, the maternal-to-fetal transport of glucose may be altered in certain situations. DiGiacomo and Hay23 have recently reported endogenous glucose production by the fetal lamb in late gestation during episodes of hypoglycemia and hypoinsulinemia. We anticipate that with the availability of cordocentesis, additional information regarding human maternal-fetal glucose metabolism will be forthcoming.

The available information on insulin sensitivity and carbohydrate metabolism in early pregnancy is controversial. Kalkhoff et al.4 believe that there is increased insulin sensitivity in early pregnancy, on the basis of improved intravenous GTTs in pregnant women during the first trimester relative to nongravid controls. Interestingly, the K values in our subjects also showed an increase, albeit nonsignificant, from 1.84 to 1.94 from the time before conception to 12 to 14 weeks' gestation. Coustan and Felig⁶ postulate that the decreased need for insulin in early gestation in insulindependent diabetic women is a function of decreased glucose availability. Knoop et al.24 ascribe enhanced maternal fat storage in early pregnancy to increased insulin secretion associated with increased caloric intake. The results of our study show that each of these women had evidence of decreased insulin sensitivity by 12 to 14 weeks' gestation; the mean decrease in the glucose infusion rate was 2.3 mg/kg fat-free mass per minute with a decrease in the range of 4.6 to 0.8 mg/kg fatfree mass per minute. We believe that the decrease in insulin sensitivity during gestation is associated with the increases in maternal body fat in early gestation that are necessary precursors for providing both fetal and maternal energy requirements in late gestation. A similar mechanism has been used to explain the early changes in the pathogenesis of type II diabetes in Pima Indians.25

The changes in insulin sensitivity during gestation in this study with the hyperinsulinemic-euglycemic clamp are not equivalent to estimates with the insulin/glucose ratio with the oral GTT or K value with the intravenous GTT. There was little or no significant change in either of these estimates of insulin sensitivity during gestation in our study subjects. This, however, may represent β error because of the relatively small number of subjects examined. Furthermore, we recognize that the methods we used estimate only peripheral insulin sensitivity. For the purposes of this report, we have assumed, as have others, that the elevated insulin concentrations during the hyperinsulinemic-euglycemic clamp suppress hepatic glucose production in these healthy normal subjects14 and that the glucose infusion rate was equated with peripheral insulin sensitivity. We are in the process of examining whether this assumption is correct by analyzing whether infused 6-6 dideuterated glucose during the hyperinsulinemic-euglycemic clamp showed complete suppression of hepatic glucose production

In summary, we report the first application of the hyperinsulinemic-euglycemic clamp in estimating the longitudinal changes in peripheral insulin sensitivity in pregnant women. We found that in nonobese normal women there is a significant 56% decrease in insulin sensitivity through 34 to 36 weeks' gestation. Of note, 39% of the total decrease in insulin sensitivity was evident by 12 to 14 weeks' gestation. Accordingly, this decrease in insulin sensitivity was associated with a 3.0-to 3.5-fold increase in insulin release by 34 to 36 weeks' gestation.

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Streptozocin-induced diabetes mellitus in the pregnant ewe

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To evaluate the effects of streptozocin on maternal pancreatic β-cell function, we administered the agent to 14 pregnant ewes at 85 to 90 days' gestation on two occasions, 4 days apart. Intravenous glucose tolerance tests were performed before the initial administration, before the second dose, and 4 weeks after the final dose of streptozocin. There was a significant elevation in maternal fasting blood glucose (82 \pm 8.1 mg/dl before streptozocin and 102.6 \pm 6.8 mg/dl after streptozocin, ρ < 0.05). Five late-gestation ewes were used as controls, and a significant elevation in fasting plasma glucose levels was found in the streptozocin-treated animals (71.4 \pm 7.1 mg/dl control vs 102.6 \pm 6.8 mg/dl after streptozocin, p < 0.05). The glucose tolerance test curves showed a significant elevation 4 weeks after streptozocin compared with before streptozocin (p < 0.05). The maternal insulin response to streptozocin demonstrated a loss of the second-phase insulin response to the glucose load after one dose of streptozocin and loss of the first phase after two doses. The fetuses of the streptozocin-treated ewes showed a significant elevation in plasma glucose level compared with that of controls (13.3 ± 0.8 mg/dl, n=5) vs 42.1 \pm 8.1 mg/dl, n=10; p<0.05, control vs streptozocin, respectively). There was a consistent trend to fetal hyperinsulinemia in the fetuses of the streptozocin-treated ewes, although this did not achieve statistical significance (3.3 \pm 0.8 μ IU/ml, n = 5 vs 9.6 \pm 2.5 μ IU/ml, n = 10; p = 0.06, control vs streptozocin, respectively). The fetal insulin/glucose ratio was preserved in the streptozocin-treated ewes. Comparison of fetal weights between the control and diabetic ewes showed a significant increase in fetal weight in the fetuses of diabetic ewes (3280 ± 46 gm in control fetuses vs 3710 \pm 54 gm in diabetic fetuses, p < 0.05). The alterations in the maternal glucose and insulin response resulting from streptozocin-induced pancreatic β-cell destruction combined with elevations in fetal glucose, insulin, and weight provides a large animal model suitable for investigation of gestational diabetes in pregnancy. (AM J OBSTET GYNECOL 1991;165:1673-7.)

Key words: Diabetes, pregnancy, ovine, streptozocin

Maternal diabetes mellitus continues to contribute significantly to perinatal mortality and morbidity. Basic science investigations of diabetes mellitus have been limited by the lack of appropriate large-animal models. Diabetes mellitus has been successfully induced in smaller animals, but these studies have been hampered by the inability to repeatedly sample the fetus.

Streptozocin, a naturally occurring nitrosourea, has been shown to induce diabetes in small research laboratory animals such as the rat, dog, and rhesus monkey.¹⁻³ Previous work by S.M.P. produced varying degrees of maternal hyperglycemia, resulting in a heterogeneous population representing a diversity of clinical states. Experimentation with different dosage

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regimens has refined the model to produce a more homogeneous animal preparation with consistent, modest glucose elevations and preservation of maternal health. In this article we are characterizing the maternal and fetal carbohydrate changes in this homogeneous animal model.

Material and methods

Fourteen pregnant Western-bred ewes of known gestational age received intravenous streptozocin (The Upjohn Co., Kalamazoo) on two separate occasions, 4 days apart, commencing at 85 to 95 days' gestation. Before each injection of streptozocin, a 3-hour intravenous glucose tolerance test with 25 gm of glucose administered as 50 ml of 50% dextrose in water was conducted. At the completion of the glucose tolerance test, streptozocin was administered to the ewe at a dose of 50 mg/kg maternal weight. Immediately before injection, the streptozocin was reconstituted in 50 ml of sterile 0.9% sodium chloride and then infused over 3 to 4 minutes via a peripheral hind limb vein. To ensure active secretion of insulin and maximal degranulation of the pancreatic β-cells, the streptozocin was injected while the animals were feeding. A third maternal in-

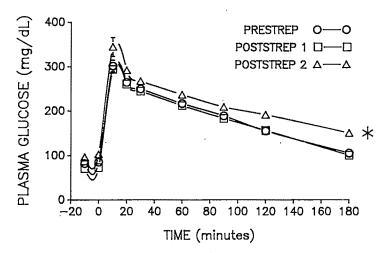


Fig. 1. Maternal glucose tolerance test profiles before streptozocin (*Pre-strep*)), after one dose of streptozocin (*Post-strep* 1), and 4 weeks after two doses of streptozocin (*Post-strep* 2) (n = 14). Data are mean \pm SEM. Asterisk, p < 0.05.

travenous glucose tolerance test was performed at 115 to 120 days' gestation.

To control for possible alterations in glucose tolerance with advancing gestation, five pregnant ewes of the same breed and supply source underwent intravenous glucose tolerance testing at 117 to 122 days' gestation.

Maternal and fetal vascular catheters were inserted with the animals under general anesthesia at 120 to 127 days' gestation, and the animals were used for other planned experimentation as approved by the University of Texas Medical School at Houston Animal Welfare Committee. Fetal plasma was sampled in both control and streptozocin-treated animals 5 to 7 days after surgery, before other planned experimentation. All experiments were conducted with the ewes standing in metabolic cages with free access to food and water. Four fetuses of streptozocin-treated ewes did not survive the perioperative period. The postoperative fetal loss rate in the streptozocin-treated ewe appears greater than that of the euglycemic ewe, an observation we attribute to the effects of maternal hyperglycemia on fetal catecholamine levels and the reduced ability of these fetuses to cope with stress (unpublished observations).

Maternal and fetal plasma glucose levels were assessed with a glucose analyzer (model 23A, Yellow Springs Instrument Co., Yellow Springs, Ohio). Maternal and fetal plasma insulin levels were measured by specific insulin radioimmunoassay (ICN Micromedic systems, Horsham, Pa.).

After the animals were killed at the completion of experimentation, weights for the fetuses of the streptozocin-treated ewes and control ewes were compared. Statistical analysis was performed by independent

Student t test and two-way analysis of variance with a computer statistical software package (Abstat, Anderson-Bell, Parker, Colo.). All data are mean \pm SE. A probability of p < 0.05 was chosen to represent statistical significance.

Results

After streptozocin administration, the fasting venous plasma glucose values rose significantly from a pretreatment level of 82.0 ± 8.05 to 102.6 ± 6.85 mg/dl 4 weeks later, p < 0.05. The glucose tolerance curves for the streptozocin-treated ewes are shown in Fig. 1. There was a significant increase in maternal plasma glucose level from baseline at all sampling intervals 4 weeks after streptozocin. Glucose values at 1 hour increased from 215 ± 7.9 mg/dl before streptozocin to 240 ± 8.0 mg/dl 4 weeks later (p < 0.05), values at 2 hours rose from 154 ± 9.1 to 191 ± 9.2 mg/dl (p < 0.05) and values at 3 hours rose from 105 ± 9.0 to 150 ± 8.5 mg/dl (p < 0.05).

The fasting plasma glucose levels of the control ewes (117 to 122 days' gestation) were significantly lower when compared with those of the gestation-matched streptozocin-treated ewes (71.4 \pm 7.1 vs 102.6 \pm 6.85 mg/dl, respectively, p < 0.05).

The serial maternal insulin responses to streptozocin are shown in Fig. 2. The maternal fasting insulin levels decreased from $22.6 \pm 2.76 \, \mu \text{IU/ml}$ before streptozocin to $12.6 \pm 2.70 \, \mu \text{IU/ml}$ 4 weeks after the second dose of the agent (p < 0.05). Within 4 days of the initial streptozocin administration there was a significant attenuation of the phase 2 insulin response, with preservation of the phase 1 response. Assessment at 4 weeks after the second dose of streptozocin revealed loss of

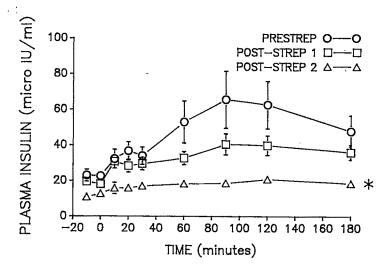


Fig. 2. Maternal insulin responses to intravenous glucose tolerance testing before streptozocin (Prestrep), after one dose of streptozocin (Post-strep 1), and 4 weeks after two doses of streptozocin (Poststrep 2) (n = 14). Data are mean \pm SEM. Asterisk, p < 0.05.

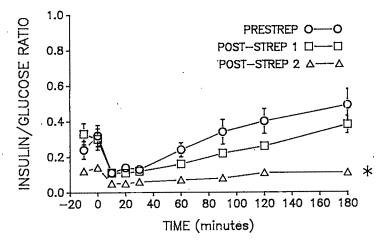


Fig. 3. Maternal insulin/glucose ratios in response to intravenous glucose tolerance testing before streptozocin (Pre-strep), after one dose of streptozocin (Post-strep 1), and 4 weeks after two doses of streptozocin (Post-strep 2) (n = 14). Data are mean \pm SEM. Asterisk, p < 0.05.

the phase 1 response and a picture of profound maternal insulinopenia with lack of insulin response to glucose challenge.

As the absolute insulin levels vary between individuals and plasma insulin concentrations should be interpreted in the context of the simultaneous glucose values, we examined the insulin/glucose ratio, a more sensitive index of pancreatic function.4 The maternal insulin/glucose ratios at the three sampling intervals are demonstrated in Fig. 3. The baseline fasting insulin/glucose ratio of 0.32 in the streptozocin-treated group was significantly reduced to 0.12 4 weeks after the second dose. The insulin/glucose ratio values were attenuated at each sampling interval throughout the glucose challenge after streptozocin.

The fetal glucose and insulin response after maternal administration of streptozocin is displayed in Fig. 4. The fetal plasma glucose levels of the streptozocintreated ewes were $42.1 \pm 8.1 \text{ mg/dl}$ (n = 10), which were significantly elevated when compared with the control fetal plasma glucose values of 13.2 ± 0.8 mg/dl (n = 5), $p \le 0.05$. The fetal plasma insulin levels of the streptozocin-treated ewes were consistently higher than those of the control fetuses, although not quite achieving statistical significance (9.62 ± 2.5 $\mu IU/ml$, $n = 10 \text{ vs } 3.3 \pm 0.8 \,\mu IU/ml$, n = 5; p = 0.06, streptozocin-treated vs control, respectively). The insulin/glucose ratio of the fetus of the diabetic ewe was 0.23, which did not differ from that of the five control fetuses, 0.25 (p > 0.05).

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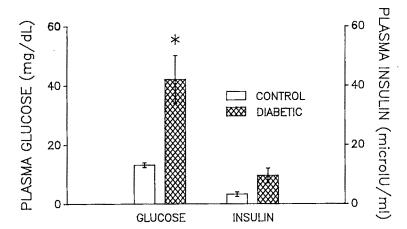


Fig. 4. Fetal plasma glucose and insulin values for streptozocin-treated ewes (n = 10) and control ewes (n = 5). Data are mean \pm SEM. Asterisk, p < 0.05.

Comparison of fetal weights between the euglycemic controls and the streptozocin-treated ewes revealed a significant increase in fetal weight at equivalent gestations in the streptozocin-treated group (3280 \pm 46 vs 3710 ± 54 gm, control vs streptozocin, p < 0.05).

Comment

The diabetogenic effect of streptozocin was first reported in 1963 by Rakieten et al. This agent has been used therapeutically in man to treat metastatic pancreatic islet cell carcinoma and in small laboratory animals to induce diabetes for research purposes. In these animal experiments, streptozocin induced a diabetic state similar to human hyperglycemia nonketotic diabetes. This effect appears to be mediated via reduction of β -cell nicotinamide adenine dinucleotide (NAD) and subsequent histopathologic alteration of pancreatic islet β -cells.

Spontaneous ovine diabetes mellitus has not been reported. The sheep is a ruminant species with little dietary intake of glucose and only small fluctuations in plasma glucose with meals. The control pregnancy ewes showed no tendency toward an elevation of plasma glucose with advancing gestation. Abnormal carbohydrate metabolism in the streptozocin-treated ewes was reflected by the presence of fasting hyperglycemia and altered glucose tolerance. This altered glucose metabolism is due to profound maternal insulinopenia, which is a secondary effect of the destructive pancreatic β -cell action of streptozocin. The phasic insulin response reflects an initial depletion of long-term stores followed by loss of short-term preformed insulin on repeat challenge.

Fetal hyperglycemia, hyperinsulinemia, and increased weight were observed. Although not reaching statistical significance, the p value is highly suggestive of fetal hyperinsulinism. Insulin is a known fetal growth hormone and in the presence of increased fetal weight

increases the significance of the elevations noted in fetal insulin levels.

Anecdotally, we have observed hyperphagia, polyuria, and polydipsia in the streptozocin-treated ewes, features that are well reported in human diabetes mellitus. Impaired tissue integrity of placental cotyledons has been noted previously by our research department in this model.⁶

Alloxan, a pancreatic islet cell toxic agent, also has been used in the pharmacologic induction of diabetes. ⁷⁻⁹ Unlike streptozocin, alloxan is not as pancreatic β-cell specific and is often toxic to other organ systems. ¹⁰ The alloxan animal preparation is both hyperglycemic and hyperketonemic because of pancreatic α-cell and β-cell destruction. Transplacental passage of alloxan, because of its small molecular size, with destruction of the fetal pancreas has been reported. ¹¹ This limits the use of this agent to acute animal studies, which do not accurately simulate the usual human disease process.

Reynolds et al.12 demonstrated that streptozocin does cross the hemochorial placenta of the rhesus monkey to a limited degree but has no discernible effect on the fetal pancreas. In the streptozocin-treated rhesus monkey, Mintz et al.18 reported fetal hyperinsulinemia and appropriate fetal pancreatic responses to glucose stimuli. We observed hyperglycemia, hyperinsulinemia, and the preservation of the insulin/glucose ratio in fetuses of diabetic ewes, implying appropriate fetal pancreatic β-cell function. Attempts at chronic studies with administration of alloxan earlier in gestation have resulted in fetal growth restriction,9 presumably as a result of fetal pancreatic destruction and secondary fetal hypoinsulinemia. The degree of fetal hyperglycemia may increase progressively with time, and studies of the fetal pancreatic response to preconception administration of streptozocin are planned.

We have demonstrated that administration of streptozocin in midgestation to the pregnancy ewe creates a

large-animal model that is useful for investigating the effects of chronic maternal carbohydrate intolerance. The maternal effects are those of hyperglycemia, insulinopenia, and an abolition of the insulin response to a glucose load. The fetus is large for gestational age, has hyperglycemia, and has an elevation in basal insulin levels, all features that are observed clinically in the fetus of a woman with gestational diabetes.

We acknowledge Wil Morgan and The Upjohn Company for their assistance in this research.

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The changing glycemic response to exercise during pregnancy

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This study was designed to test the hypothesis that pregnancy reverses the nonpregnant hyperglycemic response to sustained exercise. Serial data were obtained from 75 exercising women. Before pregnancy, exercise produced an intensity-dependent increase in blood glucose that averaged 1.5 mmol/L at high intensities. By the eighth week this response was blunted and blood glucose increased only when exercise intensity exceeded 80% of maximum. At 15 weeks this progressed and was not associated with a change in either the insulin or catecholamine response. By the twenty-third week exercise produced a decrease in blood glucose that was no longer related to exercise intensity. We conclude that the hypothesis is correct and speculate that the early change in the response is related to decreased hepatic glucose release coupled with increased glucose oxidation. In late pregnancy this is probably accentuated by fetoplacental demands. (AM J OBSTET GYNECOL 1991;165:1678-83.)

Key words: Pregnancy, exercise, glucose

The change in blood glucose level that occurs during exercise represents a shift in the balance between splanchnic glucose production and peripheral glucose uptake.¹ When production exceeds uptake, blood glucose rises, and when uptake exceeds production, it falls. In exercising men the balance between production and uptake is variable and appears to be dependent on multiple factors such as food intake, catecholamine response, physical condition, and the type, intensity, and duration of the exercise.¹-⁵ Although detailed data are not available in women, it is probable that the relationships and mechanisms are similar.

During pregnancy the amount of maternal glucose available for fetoplacental uptake varies directly with maternal levels, and in large animal models maternal hypoglycemia is associated with fetal growth restriction. Thus a maternal hypoglycemic response to regular recreational exercise during pregnancy potentially could restrict fetal glucose availability and result in some degree of fetal growth restriction.

This potential concern has been reinforced by several findings. First, data obtained in a limited number of

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subjects indicate that the consistent increase in blood glucose levels seen in response to recreational running in fit, nonpregnant women was initially blunted and then reversed after similar exercise sessions during pregnancy. Second, recreational athletes who continued a regular running or aerobics regimen in late pregnancy were delivered at term of infants with morphometric evidence of mild asymmetric growth restriction.

The current study was undertaken to explore the relationship between pregnancy and the glycemic response to exercise in greater detail. It was designed to test the hypothesis that when fit female recreational athletes continue to exercise during pregnancy the preconceptional hyperglycemic response to field exercise is reversed. The findings support the hypothesis and suggest that the pattern of food intake before, during, and after exercise may need to be altered in the recreational athlete during pregnancy.

Methods

After obtaining informed consent in accord with institutional guidelines, 75 healthy, fit, female recreational athletes who either ran (n=40) or performed aerobics (n=35) three or more times each week were enrolled for prospective study. Each subject was studied serially before conception and every 6 to 8 weeks throughout pregnancy. All subjects maintained their regular weekly exercise regimens above 60% of their preconceptional performance levels throughout pregnancy. All pregnancies were singleton, accurately dated, and clinically normal. Total pregnancy weight gain (mean 12.7 kg; range 7.3 to 17.3 kg) was adequate and agreed with the reported approximate caloric intake at the various time points of study (mean 40

kcal/kg; range 30 to 55 kcal/kg). The caloric mix was typical for an exercise populace (high complex carbohydrate, low fat, adequate protein).

To reproduce the conditions of each individual's exercise sessions outside the laboratory, all subjects were encouraged to eat their usual diet and were studied at a time relative to food intake that coincided with their regular exercise regimen (90 to 270 minutes after food intake). In 70 of the 75 subjects the study protocol began with 10 minutes of rest followed by 20 minutes of representative exercise. A free-flowing forearm venous blood sample was obtained at the end of the rest and exercise period for determination of glucose, insulin, and norepinephrine content. The exercise consisted of either a treadmill run or a standardized aerobics regimen and was conducted at a representative exercise intensity for each individual at each given time point in pregnancy. Daily exercise intensity was monitored with a portable heart rate monitor,10 and the average exercise heart rate for the preceding week was maintained during each laboratory exercise session. Exercise intensity was determined by monitoring oxygen consumption for the latter half of the exercise session and was expressed as a percentage of each individual's preconceptional maximum capacity.9

In the remaining five subjects an indwelling forearm venous sampling catheter was placed before study and free-flowing blood samples were obtained every 15 minutes throughout the protocol. The protocol began with 30 minutes of standing rest followed by a 45-minute, intensity-controlled, uphill treadmill walk and ended with 30 minutes of sitting rest. On each test occasion, the exercise intensity, determined by oxygen consumption, was maintained at a level equivalent to 50% of each individual's preconceptional maximum capacity.

Glucose levels were determined in duplicate on whole blood with a glucose oxidase method.11 Serum insulin was measured with a double antibody radioimmunoassay, modified from that described by Starr et al.,12 with interassay and intraassay coefficients of variation of <10% and <5%, respectively. Blood samples for norepinephrine were collected in chilled tubes containing ethylene glycol-bis(β-amino-ethyl ether)N,N,N',N'-tetraacetic acid and glutathione and stored at -70° C until analysis by high-performance liquid chromatography with a modification of the method of Kontur and Dawson¹⁸ (sensitivity 15 pg/ml; coefficient of variation <5%).

The data were initially grouped by the intensity of the exercise and analyzed for significant change from rest and from preconceptional values at each study point, by means of analysis of variance and Duncan's multiple range test. Linear regression was used to detect relationships between exercise intensity and the change in glucose. Significant changes over time were sought with analysis of variance and Duncan's multiple range test. Significance was set at the p < 0.05

Results

The effect of 20 minutes of running or aerobics on blood glucose levels under conditions similar to those experienced during daily exercise in the field are shown in Table I. The number of subjects exercising within a given intensity range is not shown, as it changed from one time point to another. Before conception 16 exercised below 60% of their maximum and 33 exercised above 69% of their maximum, with an overall average exercise intensity of 68% of maximum. At 8 weeks 25 exercised below 60% and 25 above 69% of their maximum, with the average overall value being 64% of maximum capacity. At 15 and 23 weeks the size of the low-intensity group was stable at 25, but only 18 exercised above 69% of maximum with mean intensities of 63% and 61% of maximum, respectively. After the thirtieth week, 29 exercised at <60% of maximum while 18 continued to exercise above 69% of maximum, and the overall mean intensity was maintained at 62% of maximum capacity.

Before pregnancy postexercise blood glucose levels exceeded preexercise values 96% of the time and the increase was significant at all exercise intensities. The average postexercise elevation varied directly with exercise intensity over a blood glucose range of 3.5 to 7.95 mmol/L at exercise intensities between 42% and 87% of maximum (change in glucose = -2.02 $\text{mmol/L} + 0.042 \times \text{percent maximum}, r = 0.5425$). This predicts that blood glucose will rise at exercise intensities in excess of 48% of maximum capacity at a rate of 0.44 mmol/L for each 10% increase in exercise intensity above that level. At <60% of maximum intensity the increase averaged 0.32 ± 0.10 mmol/L (mean \pm SEM) rising to 1.54 \pm 0.29 mmol/L at intensities >80% of maximum.

By the eighth week of gestation the response changed, with postexercise glucose levels exceeding preexercise values <40% of the time. A significant increase postexercise occurred only when intensity exceeded 80% of maximum capacity, with the average increase in blood glucose falling by 33% to 1 mmol/L. There was a significant downward shift in both the slope and the intercept of the relationship between exercise intensity and the change in blood glucose (change in glucose = $-2.04 \text{ mmol/L} + 0.034 \times \text{percent max}$ imum, r = 0.4868). This predicts that blood glucose will rise only at exercise intensities >63% of maximum capacity at a rate of 0.34 mmol/L for each 10% increase in intensity above that level. This change suggests that a shift in the relationship between hepatic glucose pro-

Table I. Blood glucose levels before and after exercise

Time	D	Postexercise level by exercise intensity (mmol/L)				
	Preexercise level at rest (mmol/L)	<60% Maximum	60%-69% Maximum	, 70%-79% Maximum	>80% Maximum	
Before pregnancy 8 wk 15 wk 23 wk ≥30 wk	4.72 ± 0.43 4.60 ± 0.44 4.56 ± 0.46 4.41 ± 0.44 4.66 ± 0.55	5.04 ± 0.92* 4.15 ± 0.61 3.96 ± 0.55* 3.88 ± 0.58* 3.96 ± 0.56*	5.17 ± 0.68* 4.59 ± 0.62 4.29 ± 0.50 3.96 ± 0.57* 4.14 ± 0.45*	$5.95 \pm 0.95*$ 4.87 ± 0.64 4.32 ± 0.32 4.17 ± 0.55 $4.16 \pm 0.47*$	6.27 ± 0.98 * 5.60 ± 0.77 * 4.83 ± 0.47 4.12 ± 0.54 4.41 ± 0.40	

Data presented as mean ± SD.

Table II. Insulin and norepinephrine levels before and after exercise

Time	Insulin ($\mu U/ml$)		Norepinephrine (pg/ml)		
	Preexercise	Postexercise	Preexercise	Postexercise	
Before pregnancy	15.9 ± 8.8	6.3 ± 4.6	508 ± 166	2180 ± 862	
3 wk	12.4 ± 9.6	6.1 ± 3.1	530 ± 189	$1545 \pm 487*$	
15 wk	15.3 ± 11.2	6.1 ± 3.2	573 ± 221	1553 ± 558*	
23 wk	15.6 ± 9.0	6.2 ± 3.9	566 ± 177	1203 ± 429*	
≥30 wk	$31.6 \pm 25.1*$	$13.7 \pm 10.3*$	494 ± 171	$1152 \pm 737*$	

Data presented as mean ± SD.

duction and peripheral use during exercise begins early in pregnancy.

In the fifteenth week the change progressed with postexercise glucose levels exceeding preexercise levels <25% of the time. Postexercise values were significantly lower ($-0.62 \pm 0.12 \text{ mmol/L}$) than those observed preexercise at intensities <60% of maximum capacity and were unchanged at all higher intensities. The regression equation (change in glucose = $-2.04 \text{ mmol/L} + 0.027 \times \text{percent}$ maximum, r = 0.4494) predicts that exercise in the fifteenth week of gestation will increase blood glucose only at intensities >76% of maximum capacity at a rate of only 0.27 mmol/L for each 10% increase in intensity above that level.

By the twenty-third week, postexercise glucose levels rose <20% of the time and a significant decrease in glucose levels of similar magnitude (-0.60 ± 0.15 mmol/L) occurred after exercise at all exercise intensities <70% of maximum capacity. Regression of exercise intensity versus the change in glucose level was no longer significant (r=0.1988) with a slope of 0.010. After the thirtieth week, postexercise glucose levels rose <10% of the time, a significant decrease in glucose levels occurred after exercise at all exercise intensities <80% of maximum capacity, and the relationship between exercise intensity and the change in blood glucose remained nonsignificant.

In the subjects who maintained their exercise intensity at <60% of maximum capacity, which approximates the recommendations of the American College

of Obstetricians and Gynecologists, ¹⁴ exercise was associated with a consistent, significant decrease in blood glucose from the fifteenth week onward. In the twenty-third, thirtieth, and thirty-seventh week >20% of the individuals had postexercise values ≤3.3 mmol/L with mean postexercise levels approximating 3.9 mmol/L.

Table II details the serial changes observed in the insulin and norepinephrine levels after 20 minutes of representative exercise during pregnancy. Unfortunately, between 10 (14%) and 16 (23%) of the sample sets were partially incomplete at each time point, but their distribution over the various intensity ranges was random. Serum insulin levels decreased significantly after exercise at all time points, and the magnitude was unchanged by increasing exercise intensity. Both preexercise and postexercise levels remained at or near those observed before conception through the twentythird week. After the thirtieth week, both the preexercise and postexercise values rose significantly above the concentrations observed before pregnancy, reflecting the development of relative insulin resistance in all subjects in late pregnancy. Preexercise norepinephrine levels did not change significantly with advancing gestation. However, the mean postexercise level fell significantly in the eighth week and remained at that level for the remainder of the pregnancy. This appeared to be due to the 4% to 7% decrease in mean exercise intensity during pregnancy. When the raw data were corrected for exercise intensity, a significant decrease from preconceptional levels was seen only after the

^{*}Significantly different from rest (p < 0.05).

^{*}Significantly different from value before pregnancy, p < 0.05.

Table III. Respiratory exchange ratio during exercise

	Exercise intensity during measurement				
Time	<60%	60%-69%	70%-79%	>80%	
	Maximum	Maximum	Maximum	Maximum	
Before pregnancy	0.910 ± 0.039	0.929 ± 0.038	0.945 ± 0.037	0.955 ± 0.041 $0.990 \pm 0.015^{*}$ 0.968 ± 0.033 0.980 ± 0.041 0.947 ± 0.040	
8 wk	0.953 ± 0.038*	0.946 ± 0.042*	0.974 ± 0.018*		
15 wk	0.944 ± 0.046*	0.983 ± 0.036*	0.976 ± 0.022*		
23 wk	0.939 ± 0.038*	0.960 ± 0.021*	0.965 ± 0.037		
≥30 wk	0.942 ± 0.031*	0.957 ± 0.028*	0.952 ± 0.019		

Data presented as mean ± SD.

Table IV. Insulin level and respiratory exchange ratio before and during exercise at 50% of maximum capacity

	Insulin (µU/ml)		Respiratory exchange ratio		
Time	Rest	Exercise	Rest	Exercise	
Before pregnancy	10.2 ± 3.9	3.6 ± 1.3	0.834 ± 0.044	0.866 ± 0.050	
8 wk	9.9 ± 4.0	3.4 ± 1.1	0.850 ± 0.029	$0.934 \pm 0.025*$	
15 wk '	10.4 ± 3.6	2.6 ± 1.6	0.834 ± 0.081	$0.912 \pm 0.024*$	
23 wk	10.9 ± 3.8	2.9 ± 1.0	0.864 ± 0.059	$0.924 \pm 0.022*$	
30 wk	26.7 ± 10.2	$6.3 \pm 2.5*$	$0.871 \pm 0.054*$	$0.921 \pm 0.026*$	

Data presented as mean \pm SD.

thirtieth week at exercise intensities >69% of maximum capacity.

Table III details the serial changes observed in the respiratory exchange ratio during exercise. It increased significantly by the eighth week at all exercise intensities, and the elevation was maintained at all intensities through the fifteenth week. Thereafter, changes in the respiratory exchange ratio became nonsignificant at the higher intensities, but a significant increase in the ratio was maintained at exercise intensities <69% of maximum capacity. With Lusk's calculation,15 the pregnancy-associated increase in the ratio approximates a 5% to 7% increase in the fractional oxidation of carbohydrate during exercise in pregnancy.

The serial changes in the five subjects who exercised at a constant intensity (50% max) throughout pregnancy were similar to those observed in the larger group. Before pregnancy, a small but significant increase in blood glucose ($+0.35 \pm 0.06 \text{ mmol/L}$) was observed by the thirtieth minute of exercise, with a gradual return to preexercise levels during recovery. In the eighth week this did not occur and by the fifteenth week the response was reversed with a significant decrease in blood glucose ($-0.62 \pm 0.08 \text{ mmol/L}$) by the fifteenth minute of exercise that persisted (-0.46to -0.64 mmol/L) for the remainder of the exercise and recovery period (60 minutes). A similar pattern was observed at 23, 30, and 37 weeks, with average decreases in blood glucose level during exercise and

recovery of -0.63, -0.56, and -0.61 mmol/L at the three respective time points of study. Norepinephrine levels at rest, during exercise, and at recovery were similar to those observed before conception until the thirtieth week. Thereafter, levels at rest and during exercise were significantly decreased (rest, 727 to 457 and 529 pg/ml; exercise, 1240 to 848 and 782 pg/ml), but the absolute change from rest to exercise was significantly decreased only at 37 weeks (change in norepinephrine, 513 to 271 pg/ml). Table IV details the serial changes in insulin and the respiratory exchange ratio during exercise in these five subjects. Again there was no significant change in insulin levels until the thirtieth week, when the levels both at rest and during exercise increased. The respiratory exchange ratio at standing rest increased significantly after the thirtieth week, but the values during exercise were increased significantly above preconceptional values throughout pregnancy ($+0.056 \pm 0.006$ units).

Comment

These serial data, gathered under conditions designed to mimic an individual's daily habits (dietary, temporal, exercise type, and intensity), demonstrate that the nonpregnant hyperglycemic response to recreational running and aerobics is initially blunted and then reversed early in the course of pregnancy. Because weight gain, caloric intake, and caloric mix were relatively constant and adequate, this suggests that some of

^{*}Significantly different from value before pregnancy, p < 0.05.

^{*}Significantly different from value before pregnancy, p < 0.05.

the metabolic adaptations induced by pregnancy alter the balance between hepatic glucose production and peripheral use, which becomes apparent only when peripheral use is increased. A similar hypoglycemic shift, the so-called accelerated starvation of pregnancy,¹⁶ is another example of this phenomenon.

While the underlying mechanism for this shift during pregnancy is unclear, the increase in the respiratory exchange ratio during exercise in early pregnancy suggests that this may be due in part to a pregnancy-associated increase in the fractional use of carbohydrate by muscle during exercise. The superimposition of the constant and ever-increasing glucose use by the growing placenta and fetus also must increase the demand for glucose during exercise in late pregnancy. 6, 7, 17 This additional demand is reflected in the increase in the respiratory exchange ratio at rest observed after the thirtieth week. The combination of increased glucose use by both exercising muscle and the fetoplacental unit probably is responsible for both the increase in the magnitude and the consistency of the hypoglycemic response during exercise in late pregnancy. In addition, the fact that there is no change in either the insulin or catecholamine response, coupled with the intensity dependence of the glycemic response reversal early in gestation, suggests that during exercise there is also a pregnancy-associated decrease in hepatic glucose production that is not apparent at rest.17 This physiologic change also should alter glucose homeostasis, favoring a fall in blood glucose levels during exercise. A definitive answer to both possibilities (a pregnancy-associated increase in fractional glucose use by exercising muscle and a decrease in hepatic glucose production during exercise) will require further serial studies with the use of stable isotope infusion.

The fact that the pregnancy-associated increase in the respiratory exchange ratio during exercise was consistently abolished at high exercise intensities with advancing gestation is an apparent contradiction and requires explanation. Unfortunately we do not have all the data necessary to identify the underlying mechanism. However, the consistency of the data suggests that it is real and not an interpretive error. The possibility that carbon dioxide retention occurred during highintensity exercise in late pregnancy (which would factitiously lower the ratio) is unlikely because minute ventilation progressively increased and expired carbon dioxide concentration decreased during pregnancy. Thus we are left with the speculation that muscle glycogen stores at rest are significantly reduced in late pregnancy (possibly because of the increasing requirements of the placenta and fetus) with a resultant depletion of muscle glycogen earlier during high-intensity

exercise. When combined with the presumed suppression of hepatic glucose production and fall in blood glucose levels, this change could easily limit glucose availability to exercising muscle earlier in the latter half of pregnancy. A definitive answer would require serial muscle biopsy in a limited number of subjects during protracted high-intensity exercise.

In terms of fetoplacental substrate availability, the data indicate that relatively brief, low- and moderateintensity recreational exercise rapidly lowers maternal blood glucose levels by 0.5 to 0.7 mmol/L on average and that the decrease persists for as much as 30 minutes after exercise. Whether an intermittent change of this degree is sufficient to significantly restrict fetal substrate supply and ultimately growth rate is currently unclear. However, the observation that this degree of running and aerobics in late pregnancy was associated with morphometric evidence of asymmetric growth restriction suggests that, coupled with other physiologic changes (such as a probable reduction in placental bed blood flow), it may have an appreciable effect that potentially has therapeutic value.7, 10 For example, >70% of the exercise-associated decrease in birth weight was due to a 220 gm reduction in body fat, suggesting that reduced fat cell number could have prophylactic value. Likewise, regular, low- to moderate-intensity exercise in late pregnancy has been shown to be as effective as insulin in maintaining euglycemia in a limited number of subjects. 18, 19 Given a larger experience, it is likely that it will also be shown to reduce birth weight, which should reduce morbidity in the large-for-gestational age infant.

Finally, it is unfortunate that there are no background data addressing the factors that influence glucose metabolism during exercise in nonpregnant women. This study suggests that fit women who exercise regularly may have a different response from men,²⁻⁵ because the response to sustained but short-duration exercise is consistently a hyperglycemic one whose magnitude is intensity dependent.

In summary, the preconceptional hyperglycemic response to running and aerobics, seen in female recreational athletes, is reversed during pregnancy. The reversal is initially intensity dependent and does not appear to be related to changes in either insulin or norepinephrine response. The data suggest that it may reflect a pregnancy-associated decrease in hepatic glucose production and a pregnancy-associated increase in fractional glucose use by muscle during exercise. In late pregnancy the increased peripheral use of glucose is further increased by the demands of the fetoplacental unit, which magnify the change in blood glucose response and probably obscure its intensity dependence.

The relative hypoglycemia associated with exercise persists for an as yet undefined period of time after exercise and may influence fetal growth.

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The changing thermal response to endurance exercise during pregnancy

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This study was designed to test the hypothesis that the thermal response to endurance exercise is altered by the thermal adaptations to pregnancy. Accordingly, rectal temperature was monitored in 18 recreational athletes before, during, and after 20 minutes of continuous exercise before conception and every 6 to 8 weeks during pregnancy. Mean exercise intensity was 64% of Vo₂ max before conception and did not change during pregnancy. However, the peak rectal temperature reached during exercise decreased by 0.3° C at 8 weeks and then fell at a rate of 0.1° C per lunar month through the thirty-seventh week. This appeared to be related to changes in resting temperature, thermal mass, sweating threshold, and venous capacitance that began early in pregnancy. These data suggest that the magnitude of any exercise-associated thermal stress for the embryo and fetus is markedly reduced by the maternal physiologic adaptations to pregnancy. (AM J OBSTET GYNECOL 1991;165:1684-9.)

Key words: Exercise, pregnancy, temperature

Female recreational runners have been shown to routinely have an increase in rectal temperature to 39° to 39.5° C during exercise,1,2 and under conditions of high environmental temperature and humidity, the increase exceeds this level at much lower exercise intensities.3 During pregnancy an increase in maternal rectal temperature to these levels places the embryo and fetus at theoretical teratogenic and metabolic risk.4 For this and other reasons, sanctioned guidelines recommend that exercise duration be limited to 15 minutes and that pulse rate be kept under 140 during pregnancy.5 However, the data currently available suggest that the thermoregulatory adaptations to both regular exercise and pregnancy maintain the level of thermal stress below the level of concern when recreational runners continue to run at higher intensities for more protracted periods of time during pregnancy.1, 6, 7

The current study was undertaken to explore these early observations through serial studies of well-conditioned recreational athletes who maintained a regular exercise regimen of running, aerobics, or cycling throughout pregnancy. It was specifically designed to

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test the hypothesis that the thermal response to endurance exercise is altered by the thermal adaptations to pregnancy.

Material and methods

Subjects. Eighteen well-conditioned recreational athletes were studied during their chosen form of exercise (eight ran, seven performed aerobics, and three cycled) before conception and every 6 to 8 weeks during the subsequent pregnancy. They were between the ages of 27 and 34 years, in excellent health, of upper-middle or upper socioeconomic status, and worked regularly outside the home. All had been exercising regularly (at least three times a week for ≥ 30 minutes) for ≥ 2 years before study, were fit at enrollment as assessed by percent body fat (range, 10% to 19%) and Vo₂max (range, 47 to 67 ml·kg⁻¹·min⁻¹), and maintained a regular exercise regimen throughout pregnancy. Exercise performance during pregnancy was monitored and averaged $85\% \pm 8\%$ (range, 68% to 104%) of preconceptional performance. All pregnancies were singleton, clinically normal, and accurately dated.

Experimental protocol. On each occasion testing was conducted 1 to 3 hours after eating, the subject wore the same exercise apparel, and body weight was obtained on a balance-beam scale to the nearest 100 gm after voiding. Then measurements of rectal temperature and oxygen consumption were obtained during an initial 10-minute period of quiet standing or sitting (cyclists only) rest and during the 20 minutes of exercise and 10 minutes of recovery that followed. Although absolute workload varied from session to session, during each exercise session the treadmill speed and grade were held constant for the runners, the cyclists biked

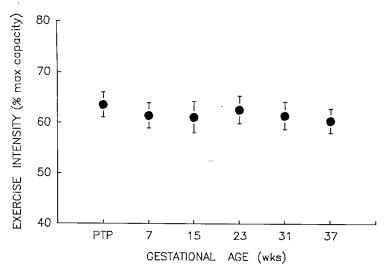


Fig. 1. Exercise intensity attained at each test time point before and during pregnancy. Data are presented as mean ± SEM. PTP, Before pregnancy.

on a Monark cycle ergometer at a constant pedal frequency and load, and the remainder followed a specially designed constant intensity aerobics routine that was displayed on a video monitor. Environmental conditions were held relatively constant for all tests (dry bulb temperature, 19° to 21° C; relative humidity, 30% to 55%; no added air flow).

Measurement techniques. Rectal temperature was monitored continuously and recorded each minute throughout the study with a precalibrated YSI thermistor (Yellow Springs Instrument Co., Yellow Springs, Ohio) with a reproducibility of $\pm 0.02^{\circ}$ C that was held in place 13 cm above the anal verge by taping it to a perineal pad. Oxygen consumption was monitored continuously throughout the last 10 minutes of exercise with a respiratory calorimetry system with a reproducibility under test conditions of $\pm 3\%$.8.9 The onset of sweating was determined by each individual's subjective sensation of sweating (occurs within 30 seconds of that determined with a resistance hygrometer sweat capsule). Exercise intensity was calculated from the mean oxygen consumption during the last 10 minutes of exercise and expressed as a percentage of Vo₂max, which was determined preconceptionally with a constant speed, progressive grade treadmill protocol.8,9

Statistics. The data were analyzed by means of repeated-measures analysis of variance and linear regression. Significance was set at the 0.01 level.

Results

As shown in Fig. 1, exercise intensity did not change significantly from one study to the next, with mean value for the group ranging between 61% and 64% of Vo₂max over the six study periods. However, the respiratory exchange ratio during exercise rose significantly (p < 0.01) from 0.86 ± 0.03 (mean ± SD) before conception to 0.91 ± 0.03 by the seventh week, representing a 1.5% increase in the caloric equivalent for oxygen. It remained near that level for the remainder of the pregnancy.

In spite of the evidence of consistent or slightly increased energy expenditure in the exercise sessions during pregnancy, the maximum rectal temperature reached during exercise (Fig. 2) fell 0.3° C by the seventh week of pregnancy and then continued to decrease linearly at an average rate of 0.1° C per lunar month. The range of individual R2s for this decremental change was 0.69 to 0.99 with a mean group R2 of 0.890 ± 0.094. This longitudinal change was significant at the p < 0.0001 level.

In addition, beginning early in pregnancy, there was a steady increase in body weight (Fig. 3) that averaged 1.7 kg per lunar month (group $R^2 = 0.994$, weight = $59.8 + 1.75 \text{ kg} \cdot \text{lunar month}^{-1}$) and a steady fall in rectal temperature at rest (Fig. 4) that averaged 0.05° C per lunar month (group $R^2 = 0.983$, temperature = 37.64° C - 0.05° C · lunar month⁻¹). Both were significant at the p < 0.0001 level. As shown in Fig. 5, the rectal temperature at which sweating began during exercise fell progressively at a rate of 0.08° C per lunar month ($R^2 = 0.993$, temperature = $37.80 - 0.09^{\circ} \text{ C} \cdot \text{lunar month}^{-1}$) and the increase in rectal temperature between rest and the onset of sweating decreased abruptly by the seventh week (0.19° to 0.06° C), as did the temperature difference between rest and the peak temperature during exercise (0.80° to 0.54° C). Again, these changes were highly significant.

Finally, before conception rectal temperature consistently began a linear increase during the first 2 minutes of exercise. This disappeared during pregnancy and actually reversed itself for the first few minutes of ex-

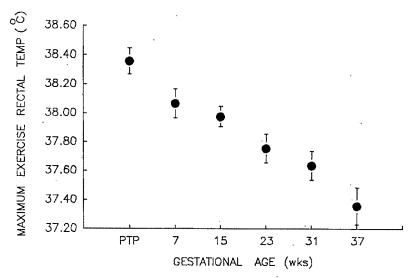


Fig. 2. Maximum rectal temperature recorded during exercise before and during pregnancy. Data are presented as mean \pm SEM. *PTP*, Before pregnancy.

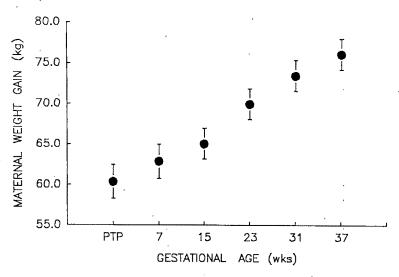


Fig. 3. Longitudinal changes in body weight during pregnancy. Data are presented as mean \pm SEM. *PTP*, Before pregnancy.

ercise beginning in the eighth week. This became more pronounced with advancing gestation. Thus in the thirty-seventh week rectal temperature fell 0.1° C at the onset of exercise and did not return to control levels until minute 8 to 10 of exercise.

The overall magnitude of the impact of these factors on the potential for embryonic and fetal thermal stress was assessed by calculating the area under the rectal temperature curve that exceeded 37.6° C during the 20 minutes of exercise and the 10 minutes of recovery and by expressing it in degree min⁻¹. The area calculated from the data obtained before conception was compared with that obtained during the seventh and thirty-first weeks of gestation. Before conception the area was 12.3 degree min⁻¹, in the seventh week the

value was only 35% (4.3 degree \cdot min⁻¹) of the preconceptional value, and it was 8% (1.0 degree \cdot min⁻¹) at 31 weeks.

Comment

These data support the initial hypothesis and indicate that multiple thermal adaptations to pregnancy alter both the magnitude of the thermal response and the peak temperature attained when continuous, moderate- to high-intensity exercise is performed during pregnancy. Furthermore, as all the changes noted appear early in pregnancy and progress, they appear to provide thermal protection for the embryo and fetus.

With one exception, these findings are similar to those reported from this laboratory earlier.¹ The ex-

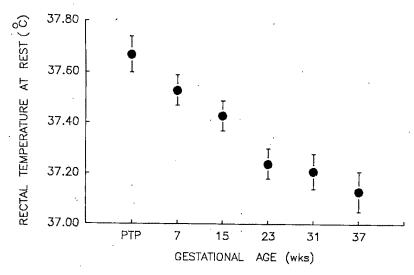


Fig. 4. Rectal temperature recorded at standing rest before exercise before and during pregnancy. . Data are presented as mean ± SEM. PTP, Before pregnancy.

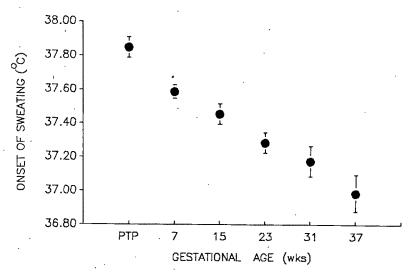


Fig. 5. Rectal temperature at which subjective onset of sweating occurred before and during pregnancy. Data are presented as mean ± SEM. PTP, Before pregnancy.

ception is that the decrease in resting temperature was not observed in the earlier study. The explanation probably lies in the fact that environmental temperature was a bit lower in the current study, which probably enhanced heat loss with a resultant lowering of rectal temperature. Indeed, other data10 suggest that it may have been lowered to the point where it actually may have induced heat generation. It is probable that either the lack of preconceptional values coupled with differences in exercise intensity or environment also explain the discrepancy between the current findings and the data obtained by Jones et al.6 in an apparently similar small group of women.

The data indicate that multiple pregnancy-associated adaptations contributed to the modified thermal response. First, maternal weight gain increased the quantity of heat necessary to raise the body temperature the same amount by 1% to 1.5% at 7 weeks and by 12% to 14% near term simply because of the thermal inertia created by the increased tissue mass.11 This increase in thermal inertia was further accentuated by the pregnancy-associated decrease in rectal temperature at the onset of exercise, discussed in detail below. Second, the progressive fall in resting rectal temperature and the more pronounced fall in the rectal temperature at which sweating began suggests that these two thermoregulatory set points decrease progressively with advancing gestation. Physiologically, these changes are not unusual, as they normally occur nocturnally12 and as part of the overall thermal acclimation to regular exercise in a hot, humid environment.7 Presently the mechanisms underlying these changes are unclear. However, they are felt to represent the normal adaptive response to changes in nonthermoregulatory metabolic heat production and have both central and peripheral components. As pregnancy is associated with an increase in metabolic rate, these changes probably represent a normal physiologic response to increased heat production. In any case, they clearly diminish the impact of any additional heat generation and improve the ability to dissipate heat.

Given that basal body temperature during pregnancy remains elevated until the mid second trimester, ^{18, 14} the fall in resting temperature in early pregnancy was a surprise. The discrepancy suggests that the decremental change was obscured in the basal body temperature studies until later in the pregnancy by the thermal insulation inherent in the "basal conditions" described. In the current study the clothing and environmental temperature offered no such insulation and would encourage heat loss, allowing early recognition of the thermal adaptations to the increased heat production of pregnancy.

The marked pregnancy-associated increase in skin temperature and blood flow,15-17 coupled with the approximate 3 L increase in minute ventilation, should have improved convective, radiant, conductive, and evaporative avenues of heat loss. Although not measured in this study, the pregnancy-associated increase in distal extremity temperature at rest is reported to exceed 4° C and hand, forearm, and calf blood flows double. All else being equal, this should have improved the efficiency of heat transfer from the body core to the skin and increase convective and radiant heat loss from the extremities by between 12% and 17%.18 However, as the pregnancy-associated change in mean skin temperature has not been measured and the distal extremities only contribute 15% to mean skin temperature, it is likely that the overall increase in convective and radiant heat loss from the skin would have been no more than 5% to 7%. The percentage increase in evaporative heat loss from the respiratory tract should have varied inversely with minute ventilation. The pregnancy-associated rise in minute ventilation should have increased respiratory evaporative heat loss between 25% and 40% at rest, but, during exercise at the levels encountered in the current study, it would have increased only 5% to 8%.

The impact of these various adaptations on overall heat dissipation at the ambient temperature used in the current study can be approximated using the partitioning of heat loss estimates obtained in nonpregnant individuals. Before pregnancy, assuming a weight of 55 kg, a rise in core temperature of 0.8° C, a power output of 700 W (10 kcal/min) from exercise and an ambient temperature of 19° to 21° C, approximately 15% (100 W) of the heat generated should be retained

to account for the increase in core temperature. Of the remainder, 40% should be lost to the environment by convection (240 W), 5% by radiation (30 W), 5% as respiratory evaporative loss (30 W), and 50% by the evaporation of sweat (300 W). During the seventh week of pregnancy the thermal adaptation of a fall in resting core temperature (0.1° C) coupled with the increase in weight (3 kg) provided an additional amount of thermal inertia which should have buffered approximately 2% (15 W) of the heat generated before core temperature returned to its preconception resting level. The remaining increase in core temperature (0.44° C) should have stored an additional 9% (63 W) of the heat produced. Thus, at 7 weeks, total maternal heat storage should have decreased by approximately 22%. The increase in skin temperature and skin blood flow should have increased convective heat loss by approximately 5% to 252 W and radiant heat loss to 32 W. Evaporative loss from the lungs should have increased approximately the same amount (32 W), whereas, in spite of the lower body temperature, evaporative loss by sweating should have increased slightly, to 306 W. This could not have occurred without the observed downward shift in the sweating threshold. By the thirty-first week the overall decrease in resting core temperature of 0.43° C, coupled with the initial 0.1° C fall in core temperature and the 14 kg increase in body weight, should have provided an overall increase in thermal inertia, which by itself would buffer approximately 13% (89 W) of the heat generated in elevating core temperature to preconception resting levels. By this time point, the efficiency of convective heat loss should have increased approximately 7% to about 257 W, with the avenues of radiant and respiratory evaporative heat loss continuing to account for approximately 64 W. As maximum core temperature only rose to the value recorded at rest before pregnancy (37.65° C%), the remainder of the power generated (290 W) should have been lost as heat through sweat evaporation. Again this was possibly due to an overall downward shift in the sweating threshold, which actually exceeded the shift observed in resting core temperature (0.68° vs 0.43° C).

Finally, the initial fall in rectal temperature at the onset of exercise during pregnancy is puzzling but suggests that an increase in venous capacitance probably occurs early in pregnancy. With this change, additional blood would pool at rest in the periphery and cool down. At the onset of exercise its return to the central circulation would decrease central temperature and effectively buffer the initial heat generated. A similar response has been observed by Hong and Nadel¹⁹ during exercise in the cold.

In summary, in fit women who continue a regular exercise program during pregnancy, the adaptations to pregnancy appear to modulate the thermal response to exercise by several mechanisms. First, a pregnant woman's thermal inertia is increased as a result of pregnancy-associated progressive decrease in resting temperature that is magnified at the onset of exercise by the return of an increased volume of cool blood from the periphery and a progressive increase in body mass. Second, a downward shift in the central thermal threshold for sweating allows evaporative heat loss to proceed at lower core temperatures. Third, the pregnancy-associated increase in skin blood flow and skin temperature enhances heat transfer from the core to the skin and increases the thermal gradient between the individual and her environment, creating a significant increase in heat loss through convection and radiation. Finally, the rise in minute ventilation slightly increases heat loss from the respiratory tract. As a result of these changes, the maximum rectal temperature attained during 20 minutes of sustained exercise that generates approximately 10 kcal/min of heat is progressively reduced. In early pregnancy the maximum rise is reduced by >30%, progressing to >70% near term.

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Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy

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In normal pregnancy the vasodilating actions of prostacyclin and the antioxidant activity of vitamin E are important for normal physiologic function. Thromboxane and lipid peroxides oppose these actions by promoting vasoconstriction and peroxidation reactions, respectively. An imbalance between thromboxane and prostacyclin and between lipid peroxides and antioxidant activity is implicated in pathologic states such as preeclampsia. We hypothesized that in normal pregnancy there would be a balance in the ratios of prostacyclin to thromboxane and of vitamin E to lipid peroxides that would favor prostacyclin and vitamin E. Blood samples were collected from normally pregnant women throughout gestation and analyzed for prostacyclin, thromboxane, vitamin E, and lipid peroxides. Serum levels of lipid peroxides remained relatively stable throughout gestation, but the levels of vitamin E progressively increased. Plasma levels of prostacyclin progressively increased with advancing gestation, whereas levels of thromboxane progressively decreased. Therefore the ratios of both prostacyclin/thromboxane, and vitamin E/lipid peroxides progressively increased during pregnancy. The increase in the ratios was highly correlated, r = 0.94. We conclude that the changes in the maternal concentrations of these compounds and the progressive increase in the ratios of prostacyclin/thromboxane and vitamin E/lipid peroxides suggest that the vasodilating actions of prostacyclin and the antioxidant activity of vitamin E are progressively favored with advancing gestation in normally pregnant women. (AM J OBSTET GYNECOL 1991;165:1690-4.)

Key words: Lipid peroxides, vitamin E, thromboxane, prostacyclin, pregnancy

Prostacyclin and thromboxane are metabolites of arachidonic acid and exert diverse biologic effects. Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation. During pregnancy it also decreases uterine contractility and increases uteroplacental blood flow. Thromboxane has the opposite effects of prostacyclin. A balance between the actions of prostacyclin and thromboxane is important in maintaining vascular function and platelet stability during normal pregnancy. An imbalance between their actions in preeclampsia is thought to play an important pathologic role in this disorder.¹

Lipid peroxides are toxic compounds that can damage enzymes, proteins, and cell membranes.^{2, 3} Lipid peroxides are related to the synthesis of prostaglandins because they are produced during the enzymatic conversion of arachidonic acid to prostacyclin, thromboxane, and other metabolites. Lipid peroxides also affect the synthesis of prostaglandins. At low concentrations

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lipid peroxides stimulate cyclooxygenase, but when their concentrations increase above normal, they inhibit prostacyclin synthase and cyclooxygenase to decrease prostacyclin synthesis. Thromboxane synthase is not affected by lipid peroxides.

The toxic actions of lipid peroxides are opposed by vitamin E, which is a free radical scavenger and thus prevents the formation of lipid peroxides. It functions as an in vivo antioxidant that protects tissue lipids from free radical attack,^{2, 7-9} thereby stabilizing cell membranes.¹⁰

Because of the opposing biologic actions of these compounds, we hypothesized that in normal pregnancy there would be a balance in the ratios of prostacyclin to thromboxane and vitamin E to lipid peroxides that would favor the concentrations of prostacyclin and vitamin E and thus presumably their biologic actions. To test this hypothesis maternal blood samples were collected throughout gestation from normally pregnant women, and the samples were analyzed for prostacyclin, thromboxane, lipid peroxides, and vitamin E.

Material and methods

Blood was collected from a total of 82 normally pregnant women and from 30 nonpregnant women. Institutional approval was obtained to conduct this study. Pregnancy was divided into 4-week intervals, and blood was collected from approximately 10 women for each interval. All subjects were between 20 and 40 years old,

and none were taking aspirin or receiving vitamin E therapy.

Venous blood samples (6.0 ml) were collected from the median cubital vein before 9 AM. Two milliliters of each sample was allowed to clot, was centrifuged, and was used as serum samples for lipid peroxide and vitamin E analysis. Four milliliters of each sample was drawn into a syringe containing 0.2 ml of 2% heparin and 0.05% indomethacin and used as plasma samples for determination of 6-keto-prostaglandin (PG) F₁₀ and thromboxane (Tx) B₂ by radioimmunoassay.

Lipid peroxides were estimated by the method of Yagi,11 which measures thiobarbituric acid-reactive products and expresses the data in terms of malondialdehyde. Four milliliters of 1/12N sulfuric acid and 0.5 ml of 10% phosphotungstic acid were added to 20 μl serum and mixed thoroughly. After centrifugation at 3000 rpm for 10 minutes, the liquid phase was decanted. Two milliliters of 1/12N sulfuric acid and 0.3 ml of 10% phosphotungstic acid were added to each sample, mixed, and centrifuged again. The liquid phase was decanted. Four milliliters of double-distilled water and 1.0 ml of TBA reagent (0.67% 2-thiobarbituric acid/acetic acid, 1:1) were then added to each sample, mixed, and heated at 95° C for 1 hour. Samples were cooled with tap water. Five milliliters of n-butyl-alcohol was added, and the samples were vigorously shaken for I minute and centrifuged. The n-butyl-alcohol phase, which contained the lipid peroxides, was used for malondialdehyde analysis with a Shimadzu FR-540 fluorospectrophotometer (Kyoto) with excitation at 515 nm and emission at 553 nm. Tetramethoxy propane (TCI, Tokyo Kasei, Japan) was used as standard and doubledistilled water as control. Recovery of exogenously added standard was 98%, and the coefficient of variation for the assay was 6.5%. Assay of varying sample volumes resulted in linear responses parallel to the standard curve.

Vitamin E was determined by fluorometric measurement of tocopherol in serum as described by Abe and Katsui.¹² One milliliter of double-distilled water and 1.0 ml of ethanol were added to 0.2 ml of serum and mixed thoroughly. Five milliliters of n-hexane was added and the samples were vigorously shaken for 1 minute. Samples were centrifuged at 1000 rpm for 5 minutes, and the hexane phase was separated and analyzed for tocopherol with a Shimadzu FR-540 fluorospectrophotometer at an excitation of 295 nm and an emission of 320 nm. DL-α-Tocopherol (E. Merck, West Germany) was used as standard and double-distilled water as control. Recovery of exogenously added vitamin E was 95%, and the coefficient of variation for the assay was 5.5%. Assay of varying sample volumes resulted in linear responses parallel to the standard curve.

Prostacyclin and thromboxane concentrations were estimated by specific radioimmunoassay of their stable metabolites, 6-keto-PGF₁₀ and TxB₂, respectively, similar to analysis with previously described assays.1 The assays consisted of competitive binding of radioactive and natural TxB₂ or 6-keto-PGF₁₀ to specific antibodies. Dextran-coated charcoal was used to separate the bound from the free fraction. Radioimmunoassay kits were obtained from the Department of Pharmacology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing). The cross-reactivity of the TxB₂ antibody was PGE₂ <0.5%, PGE₁ 0.08%, PGA₂ 0.01%, PGB₂ 0.001%, PGF_{2α} 0.17%, PGD₁ 0.43%, 6keto-PGF_{1α} 0.108%, 15-keto-PGE₂ 0.001%, and arachidonic acid 0.001%. The cross-reactivity of the 6-keto-PGF1a antibody was TxB2, PGE2, and arachidonic acid <0.03%; PGA2 1.8%; PGE1 1.5%; and PGF2a 1.2%.13 The least detectable concentration of the 6keto-PGF_{1α} assay was 12.5 pg and that of the TxB₂ assay was 6.3 pg.

Data were statistically analyzed by one-way analysis of variance and Tukey's test. A p value of <0.05 was considered to be significant.

Results

The serum concentrations of vitamin E and lipid peroxides throughout gestation are shown in Fig. 1. Vitamin E levels were significantly increased in the first trimester of pregnancy as compared with nonpregnancy (5.82 \pm 0.15 vs 5.18 \pm 0.09 μ g/ml, respectively, mean \pm SE, p < 0.01), and they progressively and significantly (p < 0.01) increased throughout the rest of gestation to $8.02 \pm 0.36 \,\mu\text{g/ml}$ at term. The levels of lipid peroxides were significantly increased in the first trimester of pregnancy as compared with nonpregnancy $(3.36 \pm 0.07 \text{ vs } 2.41 \pm 0.02 \text{ nmol/ml, respec-}$ tively, p < 0.01), and they remained significantly elevated throughout the remainder of gestation $(3.45 \pm 0.09 \text{ nmol/ml at term})$. The bottom graph of Fig. 1 shows the change in the ratio of vitamin E to lipid peroxides, which progressively increased throughout pregnancy.

The plasma concentrations of 6-keto-PGF_{1a} and TxB₂ throughout gestation are shown in Fig. 2. The level of 6-keto-PGF1a was significantly less in the first trimester of pregnancy as compared with that of nonpregnancy $(115 \pm 3 \text{ vs } 162 \pm 2 \text{ pg/ml, respectively, } p < 0.01)$, but the concentrations then increased progressively throughout the rest of gestation to 169 ± 8 pg/ml (p < 0.01). The plasma level of TxB₂ was significantly higher in the first trimester of pregnancy than in nonpregnancy (154 \pm 6 vs 102 \pm 2 pg/ml, p < 0.01). The level increased further in the early part of the second trimester (169 \pm 7 pg/ml at 12 to 16 weeks), but then levels progressively decreased during the rest of gestation (106 \pm 6 pg/ml at term, p < 0.01).

The ratio of 6-keto-PGF_{1a} to TxB₂ throughout gestation is shown at the bottom of Fig. 2. The ratio in-

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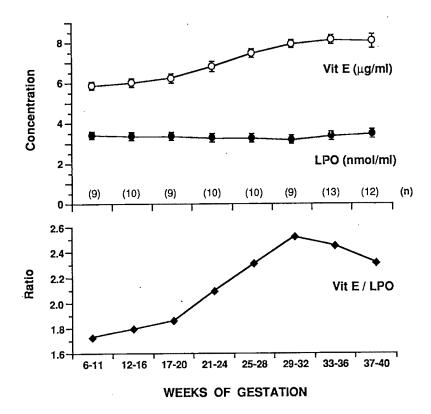


Fig. 1. Top, Maternal serum concentrations of vitamin $E(Vit\,E)$ and lipid peroxides (LPO) throughout gestation in normally pregnant women. Data represent mean \pm SE. Bottom, Ratios of mean concentrations of vitamin E to lipid peroxides throughout gestation.

creased progressively with advancing pregnancy. This increase was highly correlated with the increase in the ratio of vitamin E to lipid peroxides. The correlation coefficient was r = 0.94.

Comment

Our study is the first report of simultaneous measurements of vitamin E, lipid peroxides, 6-keto-PGF_{1α}, and TxB₂ throughout gestation and the first in which samples were collected at frequent intervals. This approach allowed us to assess not only the changes in concentrations of these compounds but also the changes in the ratios of 6-keto-PGF_{1α} to TxB₂ and of vitamin E to lipid peroxides throughout normal pregnancy. We considered the ratios to be important because of the opposing biologic actions of prostacyclin and thromboxane and of vitamin E and lipid peroxides and because imbalances in their ratios favoring the actions of thromboxane and lipid peroxides have been implicated in pathologic pregnancies, such as preeclampsia.

Maternal levels of vitamin E and lipid peroxides were both increased in pregnancy compared with nonpregnancy, which is consistent with previous reports. 14-17 This indicates that both peroxidation and antioxidation reactions are enhanced during pregnancy. The pro-

gressive increase in vitamin E levels throughout the remainder of gestation suggests there is a gradual favoring of antioxidant activity over peroxidation with advancing gestation. This also appears to be true for the placenta, in which concentrations of the antioxidants superoxide dismutase and catalase increase as gestation progresses but lipid peroxide concentrations decrease. 18, 19

The reasons for increased vitamin E levels during pregnancy are not known. Levels may increase as a physiologic response to pregnancy. For example, increased vasodilation may result in increased absorption of vitamin E from the gut. Another explanation is that vitamin E has the same carrier system in blood as cholesterol and triglycerides, the levels of which are increased during pregnancy, ¹⁶ so there may be increased binding capacity of the blood for vitamin E.

The source of increased lipid peroxides during pregnancy is unknown. Increased levels may be related to the increase in serum lipids, because serum lipids spontaneously autooxidize to form lipid peroxides. Maseki et al.²⁰ demonstrated that as the serum concentrations of total lipids increased during pregnancy, so also did the concentrations of lipid peroxides, so the ratio of lipid peroxides to total lipids did not change. Because the placenta produces lipid peroxides, it is another

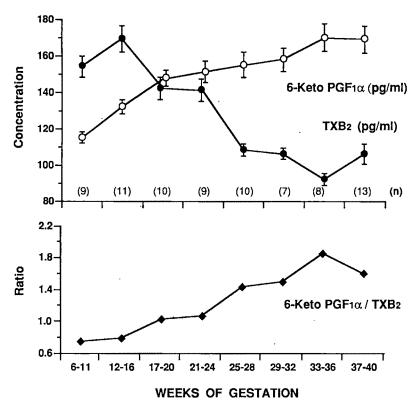


Fig. 2. Top, Maternal plasma concentrations of 6-keto-PGF_{1α} and TxB₂ throughout gestation in normally pregnant women. Data represent mean ± SE. Bottom, Ratios of mean concentrations of 6keto-PGF_{1a} to TxB₂ throughout gestation.

source.18, 19, 21 Placental lipid peroxides apparently contribute to maternal circulating levels because plasma lipid peroxide levels decrease precipitately after delivery.15

6-Keto-PGF_{1a} levels were initially lower in the first trimester than in the nonpregnant state, but then they progressively increased throughout the rest of gestation. This is consistent with reports of other investigators that 6-keto-PGF_{1α} increases with pregnancy, 22-24 although this finding differs with respect to an initial decrease in the first trimester.22,24 Plasma TxB2 levels initially increased in early pregnancy compared with nonpregnancy but then progressively declined with advancing gestation. Some investigators report an increase of TxB2 from early to late pregnancy,25 whereas others report an increase from the first to second trimesters and then a decrease, as we found.24 Previous studies determined either 6-keto-PGF1a or TxB2 concentrations at only two or three stages of pregnancy. Ours is the first study to evaluate the concentration ratios of these substances at frequent intervals throughout gestation.

As pregnancy advanced, the progressive increase in vitamin E levels in relationship to the stable levels of lipid peroxides resulted in a progressive increase in the ratio of vitamin E to lipid peroxides. Similarly, the progressive increase in 6-keto-PGF $_{\scriptscriptstyle 1\alpha}$ concentrations with

advancing gestation, along with the progressive decrease in TxB2 concentrations, resulted in a progressive increase in the ratio of 6-keto-PGF_{1a} to TxB₂. These data indicate that as normal pregnancy advances the concentration ratios progressively favor vitamin E and 6-keto-PGF_{1α}, and they suggest that the antioxidant activity of vitamin E and the vasodilating actions of prostacyclin are progressively favored with advancing gestation. This is not true in pathologic pregnancies, such as preeclampsia, where there is a reversal in these ratios to favor the vasoconstrictive actions of thromboxane and the toxic actions of lipid peroxides.26

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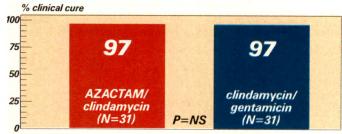
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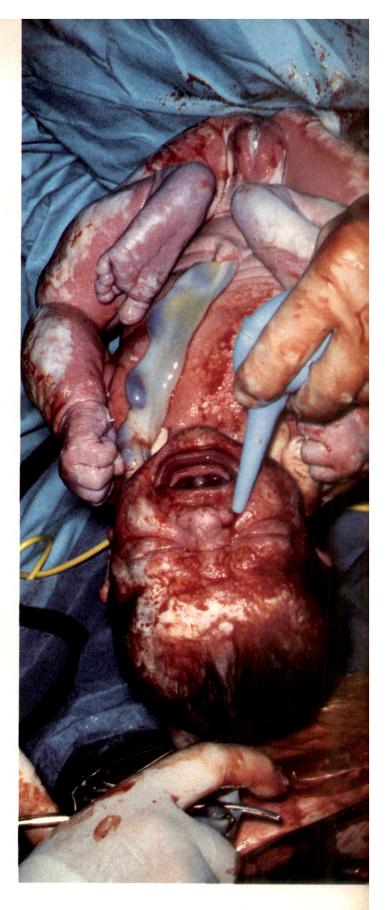


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AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowishwhite lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE—Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: Urlnary Tract Infections (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter species* and Serratia marcescens*. Lower Respiratory Tract Infections, including pneumonia and bronchitis caused by Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter species and Serratia marcescens*. Septicemia caused by Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens* and Enterobacter species Skin and Skin-Structure Infections, including those associated with postoperative wounds, ulcers and burns caused by Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter species, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Citrobacter species* intra-abdominal Infections, including peritonitis caused by Escherichia coli, Klebsiella species, including K. pneumoniae, Enterobacter species including E. cloacae*, Pseudomonas aeruginosa, Citrobacter species* including C. freundii* and Serratia species* including S. marcescens*. Gynecologic Infections, including endometritis and pelvic cellulitis caused by Escherichia coli, Klebsiella pneumoniae*, Enterobacter species* including E. cloacae* and Proteus mirabilis*.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections including appreciasion including and processor including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion including appreciasion

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gramnegative aerobic pathogens seen in general surgery.

Concurrent Therapy—Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organism(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an anti-anaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase in vitro in some gram-negative aerobes such as Enterobacter and Pseudomonas species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These in vitro findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS-Aztreonam is contraindicated in patients with known allergy to this antibiotic

WARNINGS-Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by Clostridium difficile is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin).

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS—General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility—Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed in vivo and in vitro with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy-Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers—Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use-Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS—Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity: *Hypersensitivity—anaphylaxis*, angioedema, bronchospasm. *Hematologic—pancytopenia*, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal—abdominal cramps; rare cases of *C. difficile-associated diarrhea, including pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic—purpura*, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular—hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory—one patient experienced flushing, chest pain, and dyspnea. *Hepatobililary—hepatitis, jaundice. *Nervous System—seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal—muscular aches. *Special Senses—tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other—vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole—weakness, headache, fever. *malaise.*

Adverse Laboratory Changes—Those reported without regard to drug relationship during clinical trials were: *Hepatic*—elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*—increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*—increases in serum creatinine.

OVERDOSAGE—If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION-Desage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED-AZACTAM For Injection (Aztreonam For Injection)-Lyophilized-is supplied in single-dose 15 mL viais containing 500 mg, or 1 g/viai; in single-dose 30 mL viais containing 2 g/viai; and in single-dose 100 mL intravenous infusion bottles containing 500 mg or 1 g or 2 g/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231E)

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Gynecol 65:825–829, 1985. 3. Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703–S708, 1985.



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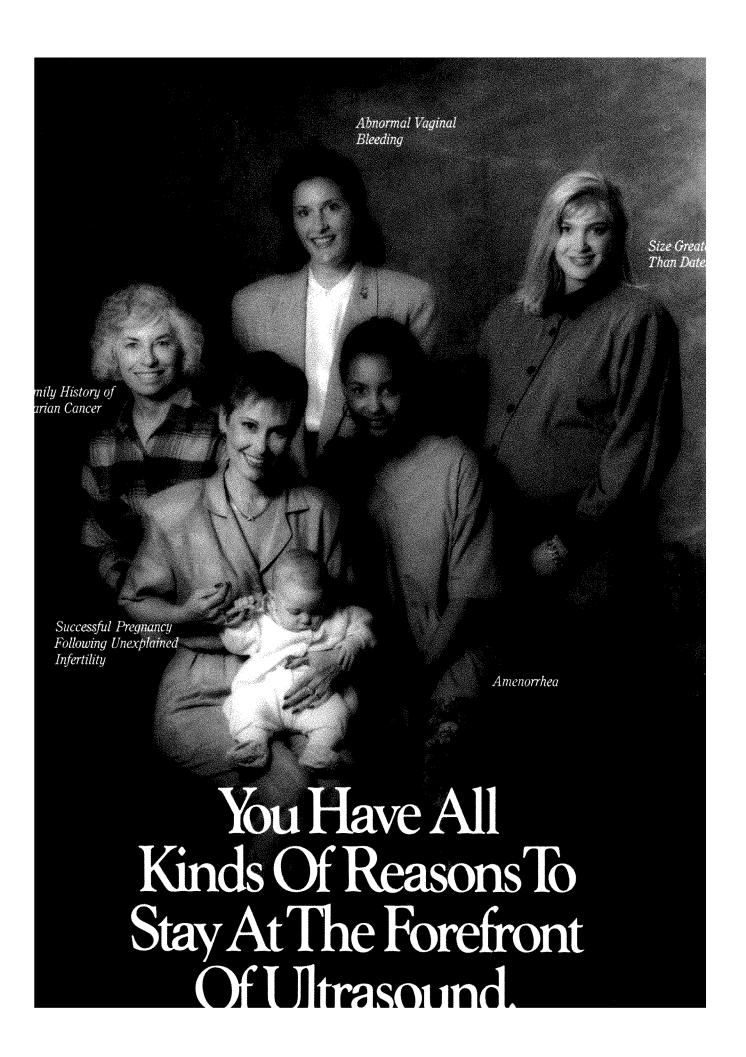
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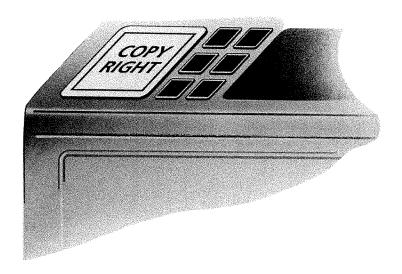
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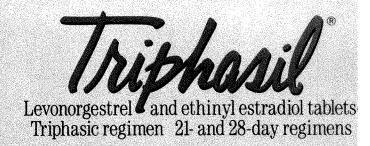
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IN BRIEF:
TRIPHASIL* — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol; 11 light-green tablets containing inert ingredients are included in the 28-day regimen) — Triphasic regimen.

Indications and Usage — TRIPHASIL* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications — OCs should not be used in women with any of the following: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Endometrial carcinoma or other known or suspected strogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contra-ceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

should be strongly advised not to smoke.

Use of OCs is associated with increased risks of serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although risk of serious morbidity/mortality is very small in healthy women without underlying risk factors. Morbidity/mortality risk increases significantly if other risk factors present (i.e. hypertension, hyperlipidemias, obesity, diabetes). Practitioners prescribing OCs should be familiar with the following information relating to these risks. (This information is based principally on data irvolving OCs with higher doses of estrogen and progestogen than those commonly used today. Effect of long-term use of lower estrogen and progestogen formulations is yet to be determined.)

1. Thromboembolic Disorders and Other Vascular Problems— MYOCARDIA, INFARCTION (M), An increased risk of MI has been attributed to OC use. Risk is primarily in smokers or women with other underlying risk factors for coronary-artery diseases (i.e. hypertension, hypercholesterolemia, morbid obesity, diabetes). Relative risk of heart attack for current OC users is estimated to be two to six, risk is very low under the age of 30. Smoking combined with OC use contributes substantially to incidence of Mis in women in their mid-thirties or older with smoking accounting for majority of excess cases. Mortality rates associated with circulatory disease increase substantially in smokers over the age of 35 and nonsmokers over the age of 40 among OC users. OCs may compound effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity in particular, some progestogens decrease HDL cholesterol and cause glucose intellerance, while estrogens may create a state of hyperinsulinism. OCs have been shown to increase blood pressure among users (see Warnings). Similar effects on risk factors are associated with increased risk of heart disease. Use OCs with caution in women with cardiovascular diseas

(see Warnings). Similar effects on risk factors are associated with increased risk of heart disease. Use OCs with caution in women with cardiovascular disease risk factors.
THROMBOEMBOLISM. Increased risk of thromboembolic and thrombotic disease associated with OC use is well established. In case control studies relative risk of users compared to non-users was 3 for first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. In cohort studies relative risk was somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. Thromboembolic disease risk due to OCs is not related to length of use and disappears after pill use is stopped.

A 2- to 4-fold increase in relative risk of postoperative thromboembolic complications has been reported with OCs. Relative risk of venous thrombosis in women with predisposing conditions is twice that of women without such conditions. If feasible, discontinue OCs at least 4 weeks prior to and for 2 weeks after elective surgery of a type associated with increased risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is associated with an increased thromboembolic risk, start OCs no earlier than 4 to 6 weeks after delivery in women not breast-feeding, or a mild-timester prognancy termination. CEREBROVASCULAR DISEASES. OCs increase relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), in general, risk is greatest among older (> 35 years). Hypertensive women who smoke, hypertensive women who smoke, hypertensive showing interacts to increase hemorrhagic stroke risk.

DOSE-RELATED RISK OF VASCULAR DISEASE FROM OCS. A positive association has been observed between amount of estrogen and progestogen in OCs and vascular disease risk. A decline in serum high density lipoproteins (HDL) is reported with many progestational agents. Seru

The dosage regimen prescribed should contain the least amount of estrogen and progestogen compatible with a low failure rate and individual patient needs. Start new acceptors on preparations containing less than 50 mcg of

estrogen.

PERSISTENCE OF RISK OF VASCULAR DISEASE. Two studies have shown persistence of vascular disease risk for ever-users of OCs. In a U.S. study, MI risk after OC discontinuation persists for at least 9 years in women 40-49 years who had used OCs for five or more years, increased risk was not demonstrated in other age groups. In a study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after OCs stopped, although excess risk was very small. Both studies used OC formulations with 50 micrograms or higher

stopped, aithough excess risk was very small. Both studies used OC formulations with 50 micrograms or higher of estrogens.

2. Estimates of Mortality from Contraceptive Use — A study using data from several sources concluded that with the exception of OC users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The possibility of increased mortality risk with age for OC users is based on data from the 1970s — but reported in 1983. However, current practice involves use of lower estrogen dose formulations combined with careful restriction of OC use to women without the various risk factors listed in this labeling.

Changes in practice and new data suggesting that cardiovascular disease risk with OCs may be less than previously observed prompted the Fertility and Maternal Health Drugs Advisory Committee to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with OC use after age 40 in healthy nonsmokers (even with newer low-dose formulations), greater potential health risks are associated with prepancy in older women and with the alternative surgical and medical procedures which may be necessary if effective, acceptable contraception is not available.

The Committee concluded that the benefits of OC use by healthy nonsmoking women over 40 may outweigh the possible risks. Older women, as all women who take OCs, should use the lowest possible effective dose formulation.

formulation.

3. Carcinoma of the Reproductive Organs — Numerous epidemiological studies have looked at the incidence of breast, endometrial, ovarian and cervical cancer in women using OCs. Overwhelming evidence suggests that OC use is not associated with an increase in risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. A few studies show a slightly increased relative risk of developing breast cancer, although the methodology of these studies show a slightly increased relative risk of developing breast cancer, although the methodology of these studies. Including differences in examination of users and nonusers, and in age at start of use, has been questioned. Some studies suggest that OC use is associated with an increased risk of cervical intraepithelial neoplasia in some populations of women. However, controversy continues about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between OC use and breast and cervical cancers, a cause and effect relationship has not been established.

relationshin has not been established

relationship has the overtestablished.

4. Hepatic Neoplasia—Benign hepatic adenomas are associated with OC use, although incidence is rare in the U.S. Indirect calculations estimate attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through introduced in the production of the intra-abdominal hemorrhage

British studies have shown an increased risk of hepatocellular carcinoma in long-term (> 8 years) OC users; these cancers are extremely rare in the U.S. and attributable risk (excess incidence) of liver cancers in OC users approaches less than one per million users.

5. Ocular Lesions — There are clinical case reports of retinal thrombosis with OC use. Discontinue OCs if there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular

is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions; undertake appropriate diagnostic and therapeutic measures immediately.

6 Oral-Contraceptive Use Before or During Early Pregnancy — Extensive epidemiological studies revealed no increased risk of birth defects when DCs used prior to pregnancy. Studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction deflects are concerned, when taken inadvertently during early pregnancy. OC-induced withdrawal bleeding should not be used as a pregnancy test. Do not use Ocs during pregnancy to treat threatened or habitural abortion. Rule out pregnancy of two consecutive periods missed before continuing OC use. If patient has not adhered to prescribed schedule, consider pregnancy at time of first missed period. Discontinue OC if pregnancy confirmed.

7. Gallbladder Disease — Earlier studies reported an increased lifetime relative risk of gallbladder surgery in users of OCs and estrogens, more recent studies show that the relative risk of developing gallbladder disease among OC users may be minimal, which may be related to use of formulations with lower hormonal estrogen and propestogen doses.

progestogen doses.

Indigential states of the state

agents), observe pictalactic and adoctor women cooledney white taking 60s. In hori dataset women, of no apparent effect on fasting blood glucose. A small proportion of women will have persistent hypertriglyceridemia while on OCs. Changes in serum triglycerides and lipoprotein levels have been reported in OC users (see Warnings).

triglycerides and lipoprotein levels have been reported in OC users (see Warnings).

9. Elevated Blood Pressure — Increase in blood pressure has been reported in women on OCs; increase is more likely in older OC users and with continued use. Data show that incidence of hypertension increases with increasing quantities of progestogens.

Encourage women with history of hypertension or hypertension-related diseases, or renal disease to use another contraceptive method. Monitor hypertensive women electing to use OCs closely; discontinue OC if significant blood pressure elevation occurs. For most women elevated blood pressure returns to normal after OC stopped. No difference in occurrence of hypertension among ever- and never-users exists.

10. Headache — Discontinue OC and evaluate cause at onset or exacerbation of migraine, or if new pattern of headache in recurrent presiphent exercises.

10. Headache.— Discontinue OC and evaluate cause at onset or exacerbation of migraine, or if new pattern of headache (i.e. recurrent, persistent, severe) develops.

11. Bleeding Irregularities — Breakthrough bleeding and spotting sometimes occur especially during first 3 months of use. Type and dose of progestogen may be important. Consider non-hormonal causes and take adequate diagnostic measures to rule out malignancy or pregnancy in event of breakthrough bleeding, as with any abnormal vaginal bleeding, if pathylogy excluded, time or a formulation change may solve the problem, in the event of amenorrhea, rule out pregnancy. Some women encounter post-pill amenorrhea or oligomenorrhea especially when such a condition was pre-existent.

Pre-existens.

Precautions

Precautions

1. Physical Examination and Follow Up — A complete medical history and physical examination should be taken prior to initiation or reinstitution of OCs and at least annually during use. Physical exams should include special reference to blood pressure breasts, abdomen and pelvic organs, including cervical cytology, and relevant abnoratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, conduct appropriate diagnostic measures to rule out malignancy. Monitor women with strong family history of breast cancer or who have breast nodules with particular care. 2 Light Disorders.—Follow women being treated for hyperilipidemias closely if they elect to use OCs. Some progestogens may elevate LDL levels and may render control of hyperilipidemias more difficult. See Warningsi 3. Liver Function.—Discontinue OC if Jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. Fluid Retention.—OCs may cause some degree of fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions possibly aggravated by fluid retention 5. Emoland Disorders.—It significant depression occurs stop medication and use afternate contraceptive method in attempts to determine if symptom is drug related. Observe carefully those with history of depression and stop drug if depression recurs to serious degree. 6. Contact Lenses—Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding and menstrail rregularities are associated with concomitant rifampin use. A similar association, though less marked, is suggested with barbiturates, phenylbutazone, phenyton sodium, and possibly with giseofluvin, amplicillin and tetracyclines. 8. Interactions with Laboratory Tests—Certain endocrine- and liver-function tests and blood components may be affected by OCs. a Increased prothombin and fa

compretely weared.

Information for the Patient — See Patient Package Labeling.

Adverse Reactions — An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis; arterial thromboembolism; pulmonary embolism; myocardial infarction; cerebral hemorrhage; cerebral thrombosis; hypertension; galloladder disease; hepatic adenomas or benign

There is evidence of an association between the following conditions and OC use, although additiona confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis.

confirmatory studies are needed: mesenteric thrombosis; retinal thrombosis. The following adverse reactions have been reported in patients on OCs and are believed to be drug-related nausea, vomiting, gastrointestinal symptoms (such as addominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorhea; temporary intertifity after treatment discontinued; edema; melasma which may persist, breast changes tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in OC users and the association is neither confirmed nor refuted; congenital anomalies; premenstrual syndrome; cataracts, optic neurits; changes in appetite; cystiris-like syndrome; headache; nenvousness; dizziness; hirsuitsm; loss of scalp hair; erythema multiforme; erythema modosum; hemorrhagic eruption; vaginitis; porphyria; impaired renal function; hemorytic uremic syndrome; Budd-Chiari syndrome; ache; changes in libido; cotilits; sickle-cell disease; cerebral-vascular disease with mitral valve prolapse; lugus-like syndromes.

valve prolapse; lupus-like syndromes.

Overdosage — Serious ill effects havenot been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits — The following noncontraceptive health benefits related to OC use are supported by epidemiological studies that largely utilized OC formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol. Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenormea. Effects related to inhibition of ovulation decreased incidence of uncidonal ovarian cysts; decreased incidence of ectopic pregnancies. Effects from long-term use: decreased incidence of other processed incidence of the breast, decreased incidence of user pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer. decreased incidence of ovarian cancer.

Dosage and Administration — For maximum contraceptive effectiveness, take TRIPHASIL* (levonorgestrel and ethinyl estradiol tablets — triphasic regimen 21- and 28-day regimens) exactly as directed and at intervals not over 24 hours.

If TRIPHASIL® is first taken later than first day of first menstrual cycle of medication or postpartum, contra-ceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.) For full details on dosage and administration see prescribing information in package insert.



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The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood

Yuping Wang, MD, a. b Scott W. Walsh, PhD, a Jingde Guo, MD, and Junyan Zhang, MD Richmond, Virginia, and Harbin, People's Republic of China

Preeclampsia is associated with an imbalance between thromboxane and prostacyclin. The cause of the imbalance is unknown. Preeclampsia sera contain cytotoxic factors that can damage endothelial cells. Lipid peroxides can damage cell membranes, so elevated levels in the mother's blood could be related to endothelial cell injury and decreased prostacyclin in preeclampsia. This study determined maternal plasma levels of thromboxane and prostacyclin and serum levels of lipid peroxides and vitamin E in women with normal pregnancy (n = 12), mild preeclampsia (n = 16), and severe preeclampsia (n = 19) between 36 and 40 weeks' gestation. In normal pregnancy the ratio of thromboxane to prostacyclin (0.63) favored prostacyclin, and the ratio of lipid peroxides to vitamin E (0.43) favored vitamin E. Prostacyclin was significantly decreased in both mild and severe preeclampsia: Thromboxane was not increased in mild preeclampsia but was significantly increased in severe preeclampsia. The ratio of thromboxane to prostacyclin was increased in mild preeclampsia (0.77) and greatly increased in severe preeclampsia (1.94). Lipid peroxides were significantly increased in mild preeclampsia and increased further in severe preeclampsia. Vitamin E levels were unaltered in mild preeclampsia but significantly decreased in severe preeclampsia. The ratio of lipid peroxides to vitamin E was increased in mild (0.52) and greatly increased in severe (1.09) preeclampsia. We concluded the following: (1) Maternal plasma prostacyclin is decreased in both mild and severe preeclampsia, but thromboxane is increased only in severe cases. (2) Lipid peroxides are significantly increased in both mild and severe preeclampsia and vitamin E is significantly decreased in severe preeclampsia. We speculate that this imbalance could result in endothelial and platelet cell damage and in decreased prostacyclin and increased thromboxane synthesis. (3) Preeclampsia is associated with an imbalance not only between thromboxane and prostacyclin but also between lipid peroxides and vitamin E in maternal blood. The imbalances progressively favor thromboxane and lipid peroxides with the increasing severity of preeclampsia, which is consistent with the clinical symptoms of this disorder. (AM J OBSTET GYNECOL 1991;165:1695-1700.)

Key words: Lipid peroxides, vitamin E, thromboxane, prostacyclin, preeclampsia

In normal pregnancy the concentration ratio of prostacyclin to thromboxane in maternal blood progressively favors prostacyclin as pregnancy advances, suggesting there is a balance between the actions of prostacyclin and thromboxane that favors the actions of prostacyclin. Prostacyclin is a potent vasodilator, an inhibitor of platelet aggregation, and an inhibitor of uterine contractility, and its combined actions would lead to an increase in uteroplacental blood flow in normal pregnancy. Thromboxane opposes these actions. It is a potent vasoconstrictor, a potent stimulator of platelet

aggregation, and a potent stimulator of uterine contractility, and its combined actions would lead to a decrease in uteroplacental blood flow. In preeclampsia there is an imbalance between thromboxane and prostacyclin that favors the actions of thromboxane.² This imbalance, in itself, can account for the major clinical symptoms of preeclampsia. However, the cause of this imbalance is not known.

In normal pregnancy there is also a change in the concentration ratio of vitamin E to lipid peroxides in maternal blood that progressively favors vitamin E with advancing gestation. Antioxidants are derived from endogenous synthesis or, in the case of vitamin E, from the diet. Antioxidants inhibit peroxidation reactions, and thereby protect enzymes, cells, and proteins from destruction by peroxides. Lipid peroxides are formed when a lipid interacts with a radical, such as the oxygen radical. Lipid peroxides are unstable but highly reactive and very damaging compounds. They stimulate peroxidation reactions and are very toxic to enzymes, cells, and proteins. 4.5

From the Departments of Obstetrics and Gynecology and Physiology, Medical College of Virginia," and the Department of Obstetrics and Gynecology, Harbin Medical College.

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December 1991

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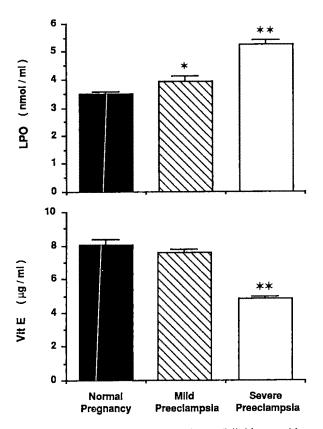


Fig. 1. Maternal serum concentrations of lipid peroxides (LPO) and vitamin E (Vit E) in normal pregnancy and in mild and severe preeclampsia between 36 and 40 weeks of gestation. Data represent mean \pm SE. Asterisk, p < 0.05; two asterisks, p < 0.01 (compared with normal pregnancy).

Rodgers et al.6 reported that preeclampsia sera contain "cytotoxic factors" that damage endothelial cells. The identity of these cytotoxic factors is not known, but lipid peroxides are likely candidates, as suggested by Hubel et al.7 Lipid peroxides enhance blood vessel contractility at doses close to the plasma levels observed in women with preeclampsia,8 and vascular responsiveness to angiotensin II is increased in vitamin E-deficient rats with increased levels of lipid peroxides.9 Even slight increases of lipid peroxides above normal can inhibit enzymes, such as cyclooxygenase and prostacyclin synthase, to decrease prostacyclin synthesis. 10-12 Larger increases damage cell membranes. 4, 5, 12 Damage to the endothelial cells in the kidneys by lipid peroxides might explain proteinuria in preeclampsia.

We hypothesize that in preeclampsia there is an imbalance of increased lipid peroxides and/or decreased antioxidants. We speculate that if there is an imbalance between lipid peroxides and antioxidants, then this imbalance might result in decreased prostacyclin synthesis, endothelial cell injury, and disruption of platelet cell membranes, leading to increased thromboxane production. The purposes of this study were to determine if there is an imbalance between lipid peroxides

and antioxidant activity in preeclampsia and, if such an imbalance exists, to determine if this imbalance is correlated with the imbalance between thromboxane and prostacyclin.

Material and methods

Blood was collected between 36 and 40 weeks' gestation from 12 women with normal pregnancy, 16 with mild preeclampsia, and 19 with severe preeclampsia. Institutional approval was obtained to conduct this study. Mild preeclampsia was defined as a blood pressure of ≥130/90 mm Hg on two separate readings at least 6 hours apart with proteinuria (<2+) or pathologic edema. Severe preeclampsia was defined as a blood pressure of ≥160/110 mm Hg and proteinuria $(\geq 2+)$ with pathologic edema. All subjects were between 20 and 40 years old, and none were taking aspirin or receiving vitamin E therapy.

Venous blood samples (6.0 ml) were collected from the median cubital vein before 9 AM. Two milliliters of each sample was allowed to clot, centrifuged, and used as serum samples for lipid peroxide and vitamin E analysis. Four milliliters of each sample was drawn into a syringe containing 0.2 ml of 2% heparin and 0.05% indomethacin and used as plasma samples for determination of 6-keto-prostaglandin (PG) $F_{l\alpha}$ and thromboxane B₂ (TxB₂) by radioimmunoassay.

Lipid peroxides were estimated by the method of Yagi,13 which measures thiobarbituric acid-reactive products and expresses the data in terms of malondialdehyde. Four milliliters of 1/12N sulfuric acid and 0.5 ml of 10% phosphotungstic acid were added to 20 μl serum and mixed thoroughly. After centrifugation at 3000 rpm for 10 minutes, the liquid phase was decanted. Two milliliters of 1/12N sulfuric acid and 0.3 ml of 10% phosphotungstic acid were added to each sample, mixed, and centrifuged again. The liquid phase was decanted. Four milliliters of double-distilled water and 1.0 ml of TBA reagent (0.67% 2-thiobarbituric acid/acetic acid, 1:1) were then added to each sample, mixed, and heated at 95° C for 1 hour. Samples were cooled with tap water. Five milliliters of n-butyl-alcohol was added, and the samples were vigorously shaken for 1 minute and then centrifuged. The n-butyl-alcohol phase, which contained the lipid peroxides, was used for malondialdehyde analysis with a Shimadzu FR-540 fluorospectrophotometer (Kyoto) with excitation at 515 nm and emission at 553 nm. Tetramethoxy propane (TCI, Tokyo Kasei, Japan) was used as standard and double-distilled water as control. Recovery of exogenously added standard was 98%, and the coefficient of variation for the assay was 6.5%. Assay of varying sample volumes resulted in linear responses parallel to the standard curve.

Vitamin E was determined by fluorometric measure-

Table I. Mean ratios of thromboxane to prostacyclin and lipid peroxides to vitamin E

	Thromboxane/ prostacyclin	Lipid peroxides/ vitamin E
Normal pregnancy	0.63	0.43
Mild preeclampsia	0.77	0.52
Severe preeclampsia	1.94	1.09

ment of tocopherol in serum as described by Abe and Katsui.¹⁴ One milliliter of double-distilled water and 1.0 ml of ethanol were added to 0.2 ml of serum and mixed thoroughly. Five milliliters of n-hexane was added and the samples vigorously shaken for 1 minute. Samples were centrifuged at 1000 rpm for 5 minutes, and the hexane phase was separated and analyzed for tocopherol with a Shimadzu FR-540 fluorospectrophotometer at an excitation of 295 nm and an emission of 320 nm. DL-α-Tocopherol (E. Merck, Germany) was used as standard and double-distilled water as control. Recovery of exogenously added vitamin E was 95%, and the coefficient of variation for the assay was 5.5%. Assay of varying sample volumes resulted in linear responses parallel to the standard curve.

Prostacyclin and thromboxane concentrations were estimated by specific radioimmunoassays of their stable metabolites, 6-keto-PGF₁₀ and TxB₂, respectively, similar to the methods previously described.2 The assays consisted of competitive binding of radioactive and natural TxB2 or 6-keto-PGF10 to specific antibodies. Dextran-coated charcoal was used to separate the bound from the free fraction. Radioimmunoassay kits were obtained from the Department of Pharmacology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing). The cross-reactivity of the TxB₂ antibody was PGE₂ <0.5%, PGE₁ 0.08%, PGA₂ 0.01%, PGB₂ 0.001%, PGF_{2α} 0.17%, PGD₁ 0.43%, 6-keto-PGF₁₀ 0.108%, 15-keto-PGE₂ 0.001%, and arachidonic acid 0.001%. The cross-reactivity of the 6-keto-PGF1a antibody was TxB2, PGE2, and arachidonic acid <0.03%, PGA₂ 1.8%, PGE₁ 1.5%, and PGF_{2α} 1.2%.15 The least detectable concentration of the 6-keto-PGF1a assay was 12.5 pg and that of the TxB2 assay was 6.3 pg.

Data were statistically analyzed by one-way analysis of variance and Tukey's test. A p value of <0.05 was considered significant.

Results

The concentrations of lipid peroxides and vitamin E for normal pregnancy (n = 12), mild preeclampsia (n = 16), and severe preeclampsia (n = 19) are shown in Fig. 1. Lipid peroxides were significantly increased in mild preeclampsia (3.94 ± 0.16 nmol/ml, mean \pm SE, p < 0.05) as compared with normal preg-

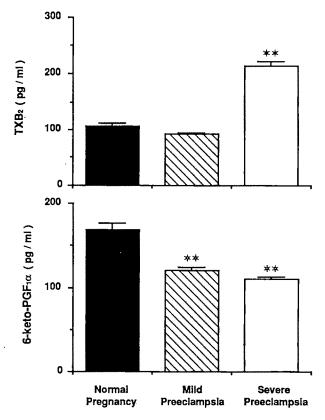


Fig. 2. Maternal plasma concentrations of stable metabolites of thromboxane and prostacyclin, TxB2 and 6-keto-PGF1a, respectively, in normal pregnancy and in mild and severe preeclampsia between 36 and 40 weeks of gestation. Data represent mean \pm SE. Asterisk, p < 0.01, compared with normal pregnancy.

nancy (3.45 ± 0.09 nmol/ml) and increased still further in severe preeclampsia $(5.26 \pm 0.15 \text{ nmol/ml})$, p < 0.01). Vitamin E levels were unaltered in mild preeclampsia (7.55 \pm 0.25 μ g/ml) and significantly decreased in severe preeclampsia (4.83 ± 0.11 μg/ml, p < 0.01) as compared with normal pregnancy $(8.02 \pm 0.36 \,\mu g/ml)$.

The concentrations of TxB2 and 6-keto-PGF1 are shown in Fig. 2. TxB₂ concentrations were not increased in mild preeclampsia (92 ± 2 pg/ml, mean \pm SE, n = 16) as compared with normal pregnancy (106 \pm 6 pg/ml, n = 12), but they were significantly increased in severe preeclampsia (213 ± 8 pg/ml, p < 0.01, n = 19). The concentrations of prostacyclin's stable metabolite, 6-keto-PGF₁₀, were significantly decreased in both mild (120 ± 4 pg/ml, p < 0.01) and severe (110 ± 3 pg/ml, p < 0.01) preeclampsia as compared with normal pregnancy $(169 \pm 8 \text{ pg/ml}).$

Table I compares the mean ratios of thromboxane to prostacyclin with lipid peroxides to vitamin E for normal pregnancy and for mild and severe preeclampsia. Both ratios increased in mild preeclampsia com-

NORMAL PREGNANCY

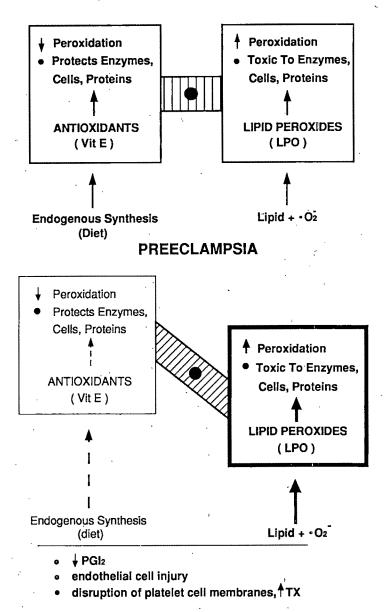


Fig. 3. Comparison of balance in biologic actions of antioxidants and lipid peroxides in normal pregnancy with imbalance of increased lipid peroxides/decreased vitamin E in preeclamptic pregnancy. Boldface type and box for lipid peroxides suggest exacerbation of their actions in preeclampsia, whereas lightface type and box for antioxidants suggest diminution of their actions.

pared with normal pregnancy and increased even further in severe preeclampsia. The relationship of the two ratios was linear (y = -0.29 + 2.04x) and highly correlated with a correlation coefficient of r = 0.997.

Comment

This is the first study to compare thromboxane and prostacyclin levels in mild preeclampsia with levels in severe preeclampsia. Maternal prostacyclin was decreased in both mild and severe preeclampsia as compared with normal pregnancy, but maternal thromboxane was increased only in severe preeclampsia (Fig.

2). The decrease in prostacyclin in preeclampsia is well documented, ¹⁶ but less information is available for thromboxane. Benigni et al. ¹⁷ also found no increase in maternal serum thromboxane levels in women with mild preeclampsia. Placental thromboxane production is significantly increased in mild preeclampsia, ² but placental thromboxane probably contributes little to maternal serum levels. The significant increase in maternal thromboxane levels we found in severe preeclampsia may be related to disruption of platelet cell membranes caused by the significant increase in lipid peroxides.

This study is also the first to compare lipid peroxide

and antioxidant levels in mild preeclampsia with levels in severe preeclampsia. Lipid peroxides were increased slightly, but significantly, in mild preeclampsia and substantially increased in severe preeclampsia as compared with normal pregnancy. Vitamin E levels were unaltered in mild preeclampsia, but markedly decreased in severe preeclampsia (Fig. 1). Other investigators have also found an increase in lipid peroxides in mild and severe preeclampsia 18-20 and a decrease in vitamin E or antioxidant activity20-22 in women with severe preeclampsia, as compared with normal pregnancy. Vitamin E was not decreased in one study of women with mild preeclampsia,23 which is consistent with our findings.

Increased thromboxane and increased lipid peroxides in preeclampsia may be coupled by the cyclooxygenase enzyme, because cyclooxygenase activity generates oxygen radicals24 that can then interact with polyunsaturated fatty acids to form lipid peroxides. Increased placental thromboxane production in preeclampsia2 may be related to the increase in lipid peroxides. The placenta is a rich source of polyunsaturated fatty acids,25 so the combination of increased oxygen radicals generated from thromboxane production with the high placental content of fatty acids would result in increased placental formation of lipid peroxides. One report indicates placental lipid peroxide autooxidation is significantly increased in preeclampsia.26

Decreased prostacyclin and vitamin E levels may be related to the increased levels of lipid peroxides. Even slight increases above normal for lipid peroxides inhibit prostacyclin synthase, and larger increases inhibit cyclooxygenase. 10-12 Thromboxane synthase does not appear to be affected by lipid peroxides.10 Still larger increases in lipid peroxides damage endothelial cell membranes, which could further impair prostacyclin synthesis.

Vitamin E is a free radical scavenger and thus exerts its antioxidant activity. However, vitamin E is consumed in exerting its action, so abnormal increases in lipid peroxides in preeclampsia could increase consumption, resulting in the decreased vitamin E levels. Another possibility is decreased absorption of vitamin E from the gut as a result of the vasoconstriction of preeclampsia.

Combining low-dose aspirin therapy to selectively inhibit thromboxane production with vitamin E supplementation to reduce lipid peroxide levels may be beneficial and further improve clinical outcomes of preeclamptic patients. Several studies indicate the positive effects of low-dose aspirin therapy for the prevention of preeclampsia, as recently reviewed,16 and at least two studies have reported beneficial effects of vitamin E supplementation or antioxidant drug therapy.27,28 Shalina et al.27 reported improvement and elimination of the momentume of areadamness with antioxidant drive therapy, but after withdrawal of the therapy the signs of the disease reappeared within 2 to 6 days.

In summary, preeclampsia is associated with an imbalance not only between thromboxane and prostacyclin but also between lipid peroxides and antioxidant activity. The imbalances are correlated and progressively favor thromboxane and lipid peroxides with the severity of preeclampsia, which is consistent with the clinical symptoms of this disorder. We speculate that the imbalance between lipid peroxides and vitamin E may result in decreased prostacyclin synthesis and endothelial cell injury in mild preeclampsia and, in addition, in disruption of platelet cell membranes leading to increased thromboxane production in severe preeclampsia (Fig. 3).

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Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension

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Increased free radical activity has been implicated in the pathogenesis of pregnancy-induced hypertension. This article investigates whether changes in antioxidant systems contribute to this condition. Two extracellular (plasma thiols and ceruloplasmin) and two intracellular (red blood cell lysate thiols and red blood cell superoxide dismutase) antioxidant markers were assayed in 25 nonpregnant women, 16 pregnant women with normal blood pressure, 19 women with pregnancy-induced hypertension, and 13 women with proteinuric pregnancy-induced hypertension. In the normotensive pregnant group (in comparison with the nonpregnant group) the plasma thiol level was reduced (p < 0.001) and the ceruloplasmin level raised (p < 0.005), suggesting increased free radical activity. The lysate thiol level increased (p < 0.005), which may reflect a compensatory protective response. In the hypertensive pregnant groups the lysate thiol rise was not present. These red blood cells may be more prone to oxidative stress. Whether this situation is a cause or an effect of oxidative stress in pregnancy-induced hypertension has yet to be elucidated. (AM J OBSTET GYNECOL 1991;165:1701-4.)

Key words: Antioxidants, free radicals, pregnancy-induced hypertension

Damage from free radicals has been implicated in many pathologic conditions. It is envisaged that increased free radical activity (oxidative stress) arises from increased production of free radicals or a deficiency in the protective antioxidant systems. Pregnancy-induced hypertension is associated with endothelial cell dysfunction. Such dysfunction could be caused by oxidative stress: the unsaturated lipids and thiol-containing proteins in cell membranes are susceptible to free radical attack. There is evidence of increased free radical activity in pregnancy-induced hypertension. but little is known about the part played by changes in specific antioxidants.

In this study two extracellular (plasma thiols and ceruloplasmin) and two intracellular (red blood cell lysate thiols and red blood cell superoxide dismutase) components of antioxidant systems have been assessed to reflect the oxidative stress across the cell membrane.

Thiol groups are involved in the defense against oxidative stress.⁶ Oxidative stress will result in the conversion of thiol groups to disulfide forms, with a consequent fall in the observed thiol level. Plasma thiols are a heterogeneous group offering a nonspecific buffer to oxidative stress. Red blood cell lysate thiols

consist mainly of glutathione, which has a specific antioxidant role.

The exact mechanism for the antioxidant ability of ceruloplasmin is uncertain. It may function by converting Fe²⁺ to Fe³⁺, Fe²⁺ being an autooxidation catalyst.⁷ Superoxide dismutase specifically detoxifies the superoxide anion radical. Because ceruloplasmin and superoxide dismutase have enzymic roles, they are not consumed by oxidative stress. Their levels, however, indicate the capacity to withstand oxidative stress and teleologically would be expected to be elevated in response to oxidative stress.

The effects of pregnancy and pregnancy-induced hypertension on these four antioxidant parameters were investigated.

Material and methods

There were four study groups: healthy, nonpregnant women in the reproductive era (n=25); pregnant women with normal blood pressure (n=16); women with pregnancy-induced hypertension without proteinuria (n=19); and women with pregnancy-induced hypertension with proteinuria (n=13). All pregnant women were primigravid and in the third trimester.

Pregnancy-induced hypertension was defined as a persistent or recurrent diastolic blood pressure of ≥ 90 mm Hg developing during pregnancy at >20 weeks' gestation, and resolving ≤ 6 weeks post partum. Proteinuria was defined as the persistent presence of protein in the urine of $\geq +$ on urine "dipstick" testing or >300 mg of protein excreted in 24 hours (this measurement was preferred where available).

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Table I. Antioxidant levels in normotensive pregnant and pregnancy-induced hypertension groups compared with nonpregnant control group

	M	Normotensive pregnant		PIH without proteinuria		PIH with proteinuria	
	Nonpregnant (mean ± SD)	Mean ± SD	p Value	Mean ± SD	p Value	Mean ± SD	p Value
Plasma thiols (U/L)	466 ± 59	292 ± 85	< 0.001	301 ± 96	< 0.001	251 ± 82	< 0.001
Ceruloplasmin (mg/100 ml)	19 ± 11	29 ± 10	< 0.005	27 ± 9	< 0.05	35 ± 8	< 0.00
Lysate thiols (U/L)	288 ± 112	453 ± 197	< 0.005	279 ± 123	NS	313 ± 115	NS
Superoxide dismutase (µg/ml)	62 ± 29	39 ± 15	< 0.005	38 ± 24	< 0.01	29 ± 16	< 0.005

p Values shown where results are of significant difference from the nonpregnant group. PIH, Pregnancy-induced hypertension; NS, not significant.

Each subject had blood sampled on one occasion. The blood was stored at 4° C and assayed within 24 hours. Preliminary studies had shown that the antioxidant levels being measured did not alter during this storage, staying within the intraassay variations (quoted below). The methods for thiol, ceruloplasmin, and superoxide dismutase estimation were described by Banford et al.8.9 Lysate thiol and plasma thiol levels were measured with the thiol-disulfide interchange reaction between 5,5'-dithio-bis(2-nitrobenzoic acid) and biologic thiols.10 Plasma ceruloplasmin activity was measured with a modification of the method of Menden et al.11 on the basis of the ceruloplasmin-catalyzed oxidation of p-phenylenediamine to Bandrowski's base. Superoxide dismutase activity was measured according to the method of Misra and Fridovich¹² on the basis of the increase in the rate of photooxidation of o-dianisidine. The intraassay and interassay variations were, respectively, 1.0% and 7.0% for lysate thiol levels, 1.2% and 1.7% for plasma thiol levels, 3.0% and 10.0% for ceruloplasmin levels, and 4.6% and 7.0% for superoxide dismutase levels.

Coincidental serum urate and blood platelet concentrations were available for the women with hypertension

Statistical significance was assessed by Student's t test.

Results

There were no significant differences in patient ages between the four study groups: nonpregnant, 26.9 ± 4.2 years (mean \pm SD); normotensive pregnant, 24.1 ± 4.0 years; pregnancy-induced hypertension without proteinuria, 24.5 ± 6.0 years; and pregnancy-induced hypertension with proteinuria, 22.2 ± 4.9 years. There were no significant differences in gestational ages between the pregnant groups: normotensive pregnant, 37.0 ± 3.3 weeks; pregnancy-induced hypertension without proteinuria, 37.8 ± 2.7 weeks; pregnancy-induced hypertension with proteinuria, 36.3 ± 3.6 weeks.

The ceruloplasmin and lysate thiol levels in the normotensive pregnant group were significantly higher and the plasma thiol and superoxide dismutase levels significantly lower than those levels in the nonpregnant group (Table I). Similar changes occurred in the hypertensive groups, except that the rise in lysate thiol levels was not found. Hence the lysate thiol levels in the pregnancy-induced hypertension groups with and without proteinuria were lower (p < 0.005 and p < 0.05, respectively) than in the normotensive pregnant group.

The only significant difference between the two hypertensive groups was that the ceruloplasmin level was higher in the pregnancy-induced hypertension with proteinuria group than in the pregnancy-induced hypertension without proteinuria group (p < 0.05).

There was a trend toward higher serum urate levels in the pregnancy-induced hypertension with proteinuria group (342 \pm 31 mmol/L mean \pm SD) in comparison with the pregnancy-induced hypertension without proteinuria group (297 \pm 83 mmol/L), but this increase did not reach significance. Blood platelet concentrations were similar in the pregnancy-induced hypertension without proteinuria (243 \pm 86 \times 10°/L) and pregnancy-induced hypertension with proteinuria (239 \pm 60 \times 10°/L) groups.

Comment

Free radicals, by their unstable and transient nature, are difficult to measure directly. Their tendency to cause lipid peroxidation¹³ has been used as an indirect measure. Markers of lipid peroxidation have been found to rise during the progression of normal pregnancy, with greater rises seen in association with pregnancy-induced hypertension.^{4, 5} Knowledge of the responses of the antioxidant systems in pregnancy and in pregnancy-induced hypertension is limited. Increasing serum antioxidant activity has been documented during normal pregnancy.¹⁴ Plasma proteins and uric acid form major components of crude antioxidant ac-

tivity. These are not dedicated antioxidants, and changes in their levels will reflect factors other than free radical attack.

In this study examination of antioxidant status in pregnancy and pregnancy-induced hypertension has been extended. Intracellular and more specific antioxidant system components have been assayed. The red blood cell was selected because it is easily accessible and may be affected in pregnancy-induced hypertension (e.g., hemolysis). It is rich in thiol functions, which are potentially involved in attack from and protection against free radicals.

Plasma thiol levels were found to be decreased and ceruloplasmin levels increased in the third trimester of normal pregnancy (compared with the nonpregnant group). Such changes are compatible with oxidative stress. The measured fall in plasma thiol concentration was greater than would be expected simply from the normal hemodilutional changes of pregnancy. Ceruloplasmin is an acute phase reactant; a rise in its level occurs with a variety of tissue insults. Thus it is difficult to attribute a cause to the elevated level, although oxidative stress is not precluded from being mechanistically involved.

The lysate thiol level was elevated in normal pregnancy. This elevation suggests either that there was less oxidative stress within the red blood cell or that the red blood cell had been provided with a higher thiol content. Both circumstances indicate that the red blood cell has increased protection against free radical attack from its environment. It is possible that such changes are required to guard the pregnant woman against a scenario of increased free radical production.

In normal pregnancy the superoxide dismutase activity was reduced. The significance of this finding is uncertain. It could reflect reduced enzyme production, implying reduced intracellular oxidative stress, or enzyme inactivation. If the cause of enzyme inactivation is related to the pathogenesis of pregnancy-induced hypertension, the superoxide dismutase activity would be expected to be lower in the hypertensive groups. Although the superoxide dismutase activity was lowest in the pregnancy-induced hypertension with proteinuria group, this low level of activity did not reach significance in comparison with the other pregnant groups. A fall in superoxide dismutase activity has also been recorded with rheumatoid arthritis9 and Graves' disease.15

In the pregnancy-induced hypertension without proteinuria and pregnancy-induced hypertension with proteinuria groups the lysate thiol amounts were significantly lower than those seen in normal pregnancy. It is anticipated that such red blood cells would be more susceptible to oxidative stress. Whether this lower lysate thiol level is a cause or an effect of pregnancy-induced hypertension cannot be concluded from this study.

The ceruloplasmin level was higher (p < 0.05) in the pregnancy-induced hypertension with proteinuria group than in the pregnancy-induced hypertension without proteinuria group. This difference could reflect greater oxidative stress being present in more severe pregnancy-induced hypertension.

It has been postulated that red blood cell dysfunction is fundamental in the development of pregnancyinduced hypertension,16 and we found antioxidant changes in the red blood cell. Similar alterations in the balance of the thiol system could be present across the endothelial cell membrane. The superoxide anion can inactivate endothelium-derived relaxing factor17 and inhibit prostacyclin synthesis.18 Lipid peroxidation products may inhibit prostacyclin synthesis 19 and stimulate smooth-muscle contraction.20 Therefore increased free radical activity may cause platelet aggregation and vasospasm, features of pregnancy-induced hypertension.

The sample sizes in this initial study are small, and the results are susceptible to β -type errors. However, changes in the antioxidant systems in normal pregnancy and in pregnancy-induced hypertension have been demonstrated. In normal pregnancy there is evidence for increased oxidative stress in the plasma compartment, but with raised red blood cell thiol defenses. In pregnancy-induced hypertension these red blood cell defenses are lowered or overcome.

The altered balances of thiol groups across the red blood cell membrane are to be investigated further with in vitro studies of intact red blood cells from subjects with pregnancy-induced hypertension.

Studies in early pregnancy are being performed to establish when the antioxidant changes of normal pregnancy occur, and whether a pattern of predictive pregnancy-induced hypertension exists.

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Editors' note

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Selective effects of preeclamptic sera on human endothelial cell procoagulant protein expression

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Current concepts of preeclampsia suggest that dysfunction of maternal vascular endothelium in vivo is a central pathogenetic feature of this syndrome. This hypothesis is suggested by the activation of the coagulation cascade associated with preeclampsia and evidence for a role of endothelium in maintaining thromboresistance. Previous in vitro studies with monolayers of human umbilical vein endothelial cells demonstrated direct cytotoxic effects of sera from preeclamptic parturients. In the current studies, we have examined the in vitro expression of three procoagulant protein activities regulated by endothelial cells: cellular fibronectin, an important mediator of platelet aggregation known to be elevated in preeclamptic women in vivo; tissue factor, the most potent endogenous procoagulant activity; and von Willebrand factor, a major component of coagulation factor VIII. Monolayer cultures of human umbilical yein endothelial cells were incubated with pregnancy sera for 24 hours before these proteins and activities were quantified. Exposure of identical endothelial cell cultures to predelivery preeclamptic sera caused significantly greater release of cellular fibronectin than postdelivery preeclamptic or predelivery or postdelivery normal pregnancy sera (p < 0.05). However, neither tissue factor activity nor von Willebrand factor expression appeared to be increased preferentially by preeclamptic sera. The data indicate that sera from women with preeclampsia induce a selective, but not a generalized, activation of endothelial cell procoagulant protein production. (AM J OBSTET GYNECOL 1991;165:1705-10.)

Key words: Preeclampsia, endothelial cell activation, procoagulant

Preeclampsia is a multisystemic, pregnancy-specific disorder defined clinically as a syndrome of elevated blood pressure, excessive proteinuria, and generalized edema.¹ Because of the associated signs of activation of the coagulation cascade and increased sensitivity to vasopressor agents, we have proposed that maternal vascular endothelial cell injury or activation is central to the development of the preeclamptic syndrome.² This hypothesis is supported by recent findings that have elucidated the role of endothelial cells in the balance between anticoagulant and procoagulant activity expression at the vessel wall. The syndrome of disseminated intravascular coagulation is a severe and rare clinical complication of sepsis and preeclampsia. However, local intravascular clotting and occlusion may oc-

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cur by subtle forms of immune or inflammatory activation, such as have been proposed in preeclampsia.¹

Previous studies designed to assess the direct effects of preeclamptic sera on human endothelial cells in vitro demonstrated an increase in chromium 51 release,3 a nonspecific indicator of endothelial cell injury, and the stimulation of platelet-derived growth factor synthesis.4 This report describes our examination of the in vitro expression of three regulated procoagulant protein activities in endothelial cells. Cellular fibronectin is an endothelium-derived isoform of fibronectin, a highmolecular-weight extracellular matrix glycoprotein involved in several fundamental cell functions. Of particular relevance to preeclampsia, cellular fibronectin mediates platelet adherence and aggregation at sites of endothelial cell injury.5 Tissue factor, a procoagulant protein synthesized and secreted by endothelial cells, is believed to be the most potent endogenous thrombogenic agent in man.6 von Willebrand factor is an endothelium-derived component of the factor VIII antigen complex, that is known to be deposited in areas of endothelial cell injury, where it mediates platelet adhesion at these sites.7 To test directly the hypothesis that a blood-borne factor(s) in women with preeclampsia modulates endothelial cell procoagulant expression, human endothelial cell monolayers were exposed to matched normal and preeclamptic pregnancy sera in vitro and assayed for altered expression of these throm-

Table I. Diagnostic criteria of preeclampsia (after Chesley⁸)

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No history of renal or cardiovascular disease
Nulliparous
Blood pressure increase from <20 wk of
≥30 torr systolic
or
≥15 torr diastolic
or
≥20 torr mean arterial pressure
Proteinuria
≥30 mg/dl (1+) on catheterized specimen
or
≥300 mg/24 hr
Hyperuricemia
≥5.5 mg/dl at term
or
≥1 SD from normal mean for earlier gestational age<sup>9</sup>
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All clinical and laboratory abnormalities must return to normal by 12 weeks post partum.

bogenic proteins. We report that serum obtained from preeclamptic women causes increased cellular fibronectin release from human umbilical endothelial cells in vitro, but effects on tissue factor and von Willebrand factor were not observed.

Material and methods

Pregnant women registering for prenatal care at Moffitt-Long Hospital and San Francisco General Hospital of the University of California, San Francisco and the University of Mississippi Medical Center were invited to participate in a prospective study of preeclampsia. Participating patients gave written consent for blood sampling in the late third trimester and again within 48 hours of delivery, as approved by the universities' committees on human research. Blood samples were collected in sterile tubes, and serum was prepared and frozen at -70° C for later use. For the predelivery specimens blood was collected before intravenous hydration, magnesium sulfate infusion, or antihypertensive medication and thus was unaffected by differences in the clinical management or therapy of parturients believed to have preeclampsia.

The patients selected for this case-control study were monitored throughout pregnancy, including labor and delivery at term (≥36 weeks' gestation) and for 12 weeks post partum with serial clinical observations. At the completion of their final postpartum evaluation, the clinical and laboratory data were reviewed by a jury of clinical investigators. Nulliparous patients were assigned to the preeclamptic study group on the basis of the recommendations of Chesley, as described in Table I. Criteria that resulted in the exclusion of patients from this study were the identification of essential hypertension or other metabolic diseases, evidence of illicit drug use by urine toxicology screens, or the failure of elevated blood pressure or proteinuria to resolve within

12 weeks of delivery. Normal controls were matched as closely as possible for parity, maternal age, and gestational age at delivery to the preeclamptic cases.

Human umbilical vein endothelial cells³ and human renal microvascular endothelial cells4 were harvested and cultured as described previously. Monolayers were grown to confluence with M-199 tissue culture media supplemented with 20% fetal bovine serum on gelatincoated Falcon 24- or 96-well plates (Becton-Dickinson, Lincoln Park, N.J.). The cell cultures were made quiescent'in serum-free M-199 media containing 500 µg/ml bovine serum albumin, 5 µg/ml transferrin, 1 µg/ml insulin, and 2 U/ml heparin for 24 hours before serumfree media containing endotoxin or 10% prepartum or postpartum human pregnancy sera were added. After an additional 24 hours' incubation the cells were assayed for tissue factor activity or von Willebrand factor as described below. Cells from representative wells were trypsinized and counted with a hemocytometer to allow normalization of procoagulant protein concentration or activity on a per cell basis.

Conditioned media were collected, brought to a final concentration of 1% aprotinin, and frozen at -70° C until assays for cellular fibronectin were performed. Cellular fibronectin levels were determined by a specific enzyme-linked immunosorbent assay (ELISA) technique with the monoclonal antibody A134, which recognizes a conformational epitope near the ED-B domain of cellular fibronectin.10 Aliquots of 100 µl of endothelial cell-conditioned medium were thawed at 37° C, diluted 1:2 with sample buffer, and assayed in duplicate. The accumulation of A134 cellular fibronectin in the endothelial cell culture supernatant was linear over 72 hours. Normal term pregnancy plasma contains cellular fibronectin in concentrations ranging from 1 to 9 µg/ml with greater levels found in preeclamptic women (range 2 to 21 µg/ml).10 However, this protein precipitates from serum during clot formation. When the 10% pregnancy serum-containing media were assayed by ELISA, no cellular fibronectin (<0.1 μg/ml) was detected in either normal or preeclampsia serum-reconstituted media. The performance characteristics and specificity of this assay, which was developed and performed at Adeza Biomedical, Sunnyvale, Calif., have been described previously.¹⁰

Tissue factor activity was measured directly in intact cells with a modification of the method of Zuckerman and Suprenant.¹¹ To each well of washed human umbilical vein endothelial cells were added 1 U each of factors VII and X (F-6509 and F-4634, respectively, Sigma Chemical Co., St. Louis). After 15 minutes' incubation at room temperature, a 1 mmol/L solution of the chromogenic substrate S-2222 (Sigma) was added to the cell supernatant, and the reaction was quantified kinetically for 4 minutes at 405 nm using

Shimadzu UV-160 spectrophotometer (Shimadzu Corp., Kyoto).

von Willebrand factor expression was assayed directly on fixed endothelial cells with a whole-cell ELISA technique. Human umbilical vein endothelial cells were exposed to pregnancy serum containing or control media with or without endotoxin for 24 hours, washed gently with M-199 media, and fixed in 2% paraformaldehyde-cacodylate buffer (pH 7.4) for 1 to 2 hours at 4° C. The cells were then washed three times with M-199 containing 5% fetal bovine serum and incubated with rabbit antihuman von Willebrand factor antisera (Dako, Santa Barbara, Calif.) at a 1:3200 dilution, which prior studies revealed to yield an optimal signal/noise ratio at 30 minutes' reaction time. Goat antirabbit immunoglobulin G conjugated to alkaline phosphatase (Vector Labs, Burlingame, Calif., 1:250 dilution) was incubated for 60 minutes and p-nitrophenyl phosphate disodium substrate (0.7 mg/ml, No. S-104, Sigma) was allowed to react for 30 minutes. The final reaction product was quantified with a microplate reader (model 3550, Bio-Rad, Richmond, Calif.) at 405 nm.

For all three assays, exposure of human umbilical vein endothelial cells to serum-free media alone and media containing endotoxin (lipopolysaccharide from Escherichia coli serotype 055:B5, 1 µg/ml, No. L-4005, Sigma) provided negative and positive controls respectively, for endothelial cell activation. Cellular fibronectin concentrations in the conditioned media are expressed as micrograms released per milliliter per 106 cells. Tissue factor activity is expressed as the change in optical density units in milliunits per minute per 106 cells. von Willebrand factor expression is presented as optical density units per 30 minutes per 106 cells. The experimental data are reported as the group mean ± SE. Paired and unpaired Student t tests were used in appropriate experiments, and conservative, nonparametric Mann-Whitney tests were used to compare the two groups of patients. Two-tailed analyses with p < 0.05 were considered significantly different.

Results

Nine women who met the requirements for the diagnosis of preeclampsia (Table I) were matched to nine normal, control parturients. The results in Table II show the mean \pm SE values for each of the clinical and biochemical parameters examined at term. As expected from diagnostic critera, patients defined as having preeclampsia had significantly greater mean arterial blood pressure, proteinuria, and uric acid levels than the normal parturients had (p < 0.05, Mann-Whitney tests); otherwise, the cases and controls were well matched (Table II).

To establish a positive control for the activation of

Table II. Demographic, clinical, and biochemical features of matched preeclamptic and normal parturients

	Preeclamptic (n = 9)	Normal $(n = 9)$	p*
Maternal age at delivery (yr)	26 ± 1	29 ± 2	NS
Parity	0	0.4 ± 0.2	NS
Gestational age at delivery (wk)	39 ± 1	39 ± 1	NS
Infant weight (gm)	3041 ± 181	3234 ± 197	NS
Placental weight (gm)	640 ± 80	742 ± 35	NS
Hematocrit (vol/vol %)	35 ± 1	36 ± 1	NS
Platelet count (×1000/mm³)	248 ± 78	219 ± 28	NS
Serum creatinine (mg/dl).	0.8 ± 0.1	0.7 ± 0.1	NS
Mean arterial pressure (torr)	112 ± 3	81 ± 2	< 0.01
Proteinuria (mg/dl)†	>30-100	<30	< 0.01
Serum uric acid (mg/dl)	6.3 ± 0.3	4.1 ± 0.5	< 0.05

NS, Not significant.

monolayer cultures of human umbilical vein endothelial cells, we incubated these cells in serum-free medium in the presence of endotoxin (1 µg/ml). Other experiments in our laboratory have demonstrated that "activated" functional and morphologic changes in human umbilical vein endothelial cells can be induced by exposure to endotoxin and interleukin-1β (unpublished observations and as described by others12). By paired analyses 1 µg/ml endotoxin induces significant increases in measurable cellular fibronectin, tissue factor, and von Willebrand factor as compared with matched human umbilical vein endothelial cell cultures without endotoxin treatment (p < 0.01, Table III).¹¹ The expression of cellular fibronectin by cultured adult human renal microvascular endothelial cells4 (1.4 μg/ml/106 cells), grown under similar serum-free conditions for 24 hours, indicates that fibronectin molecules bearing the A134 epitope are not restricted to cells of fetal origin. The specific details of each experiment are described below.

Previous studies of human umbilical vein endothelial cell perturbation had demonstrated that 10% dilutions of pregnancy sera gave reproducible and discriminating effects when normal and preeclamptic samples were compared.^{8, 4} In the experiments decribed in Table III, human umbilical vein endothelial cells were incubated with 10% predelivery and postdelivery sera from the same patients. Conditioned media were re-

^{*}These data (expressed as mean \pm SE) were analyzed by Mann-Whitney tests.

[†]Semiquantitative dipstick analysis of catheterized urine samples.

Table III. Effects of endothelial cell stin	nulation on cellular f	fibronectin, tissue fact	or activity, and
von Willebrand factor expression in vitr	· o		

Media and serum	Cellular fibronectin (µg/ml/10 ⁶ cells)	Tissue factor (mU/min/10 ⁶ cells)	von Willebrand factor (U/30 min/10 ⁶ cells)
Control	0.9 ± 0.2	35 ± 7	9.6 ± 1.1
Control plus endotoxin	1.2 ± 0.1	630 ± 38	24.6 ± 1.5
<i>p</i> *	<0.01, n = 4	<0.01, n = 5	<0.01, n=3
Normal predelivery	1.0 ± 0.1	24 ± 8	9.7 ± 1.3
Normal postdelivery	1.1 ± 0.2	21 ± 8	9.0 ± 1.0
<i>p</i> *	0.25, n = 6	0.70, n = 5	0.54, n = 6
Preeclamptic predelivery	1.6 ± 0.2	30 ± 14	9.0 ± 0.8
Preeclamptic postdelivery	1.1 ± 0.2	42 ± 16	9.2 ± 0.7
p*	0.04, n = 6	0.47, n = 5	0.77, n = 6

Data are presented as mean \pm SE of number of individual experiments (n).

moved from the cell cultures and assayed with cellular fibronectin ELISA described above. Cells exposed to 10% normal predelivery sera released 1.0 \pm 0.1 μg cellular fibronectin per milliliter per 10⁶ cells (mean ± SE, n = 6) into their conditioned media, whereas conditioned media from identical cells treated with 10% normal postdelivery sera contained 1.1 ± 0.2 μg cellular fibronectin per milliliter per 10^6 cells (n = 6, p = 0.25, paired t test). Although these results failed to reveal differences between normal prepartum and postpartum sera, experiments with 10% sera from patients with preeclampsia did demonstrate differences between paired predelivery and postdelivery samples. Cells exposed to 10% predelivery preeclampsia sera released $1.6 \pm 0.2 \,\mu g$ cellular fibronectin per milliliter per 10^6 cells (n = 6) into the conditioned media, whereas conditioned media from identical cells treated with 10% postdelivery preeclamptic sera contained 1.1 ± 0.2 μg cellular fibronectin per milliliter per 10^6 cells (n = 6, p = 0.04, paired t test, Table HI). In a single experiment exposure of human renal microvascular endothelial cells to paired predelivery and postdelivery preeclamptic serum resulted in the release of 2.0 and 0.9 μg cellular fibronectin per milliliter per 106 cells, respectively.

Stimulation of human umbilical vein endothelial cell tissue factor activity was assessed with a chromogenic substrate assay. As described above, 1 µg/ml endotoxin induced an 18-fold increase in tissue factor activity. Predelivery normal sera exposure stimulated 24 ± 8 mU, whereas paired postdelivery sera resulted in tissue factor activity of 21 ± 8 mU/min/ 10^6 cells (n = 5, p = 0.70). Similarly, paired prepartum and postpartum preeclamptic sera had insignificant effects on human umbilical vein endothelial cell tissue factor activity (30 ± 14 vs 42 ± 16 mU/min/ 10^6 cells, respectively [n = 5, p = 0.47, paired t test], Table III).

Expression of endothelial cell von Willebrand factor was quantified with a whole-cell ELISA method. Human umbilical vein endothelial cells treated with endo-

toxin as a positive control increased by 2.6-fold their concentration of cell surface von Willebrand factor protein. However, paired analyses of predelivery and post-delivery sera from normal $(9.7 \pm 1.3 \text{ vs } 9.0 \pm 1.0 \text{ U}/10^6 \text{ cells } [n=6, p=0.54, \text{ paired } t \text{ test}])$ and pre-eclamptic $(9.0 \pm 0.8 \text{ vs } 9.2 \pm 0.7 \text{ U}/10^6 \text{ cells } [n=6, p=0.77, \text{ paired } t \text{ test}])$ patients revealed no differences in von Willebrand factor expression (Table III).

To assess whether absolute differences between predelivery preeclamptic and normal sera on human umbilical vein endothelial cellular fibronectin release could be demonstrated, additional experiments were performed and combined with the previous data to provide nine patients in each diagnostic group (Table II). Human umbilical vein endothelial cells exposed to 10% predelivery preeclamptic serum released 1.8 ± 0.1 μg cellular fibronectin per milliliter per 10^6 cells (n = 9)into the conditioned media. When cultures of the same cells were exposed to 10% normal prepartum pregnancy sera, the conditioned media contained 1.3 ± 0.1 μ g cellular fibronectin per milliliter per 10° cells (n = 9, Fig. 1). Analyses of these data with nonparametric (Mann-Whitney test, p = 0.01) or parametric (unpaired Student t test, p < 0.01) statistics demonstrated significant differences between the two diagnostic groups.

Comment

A panoply of clinical and biochemical findings relevant to endothelial cell function has been demonstrated in patients with clinical preeclampsia. The increased rate of disappearance of circulating Evans' blue dye, ¹³ decreased levels of prostacyclin, ¹⁴ pathognomonic renal biopsy findings, ¹⁵ and sensitive indicators of activation of the coagulation cascade ¹⁶ all suggest that vascular endothelial cell dysfunction occurs in patients with this disease. Previous studies that specifically focused on the expression of procoagulant proteins in vivo have documented that von Willebrand factor (factor VII—related antigen)¹⁷ and plasma cellular fibronectin

^{*}p Value for paired Student t test.

concentrations10, 18 are elevated in women with preeclampsia, even before the clinical manifestations of the syndrome.

This investigation was designed to determine if endothelial cell procoagulant activation could be used as a marker of cell perturbation in an in vitro model of preeclampsia. The results demonstrate that predelivery preeclamptic sera contain a factor(s) that stimulates the secretion or release of human endothelial cell cellular fibronectin to a greater extent than sera from normal pregnancies and suggest that the clinical observation of elevated plasma cellular fibronectin concentrations in preeclamptic women^{10, 18} might be due to the in vivo release of endothelial cell-derived cellular fibronectin by this factor(s). However, in this model of endothelial cell activation neither tissue factor nor von Willebrand factor expression appears to be increased preferentially by preeclamptic sera.

The present in vitro studies contrast with the elevated von Willebrand factor activity reported in the blood of preeclamptic women.17 Because we did not measure the release of von Willebrand factor into media, it is possible that increased release was not mirrored by increased surface expression of this factor. It is also possible that different experimental conditions, e.g., exposure of the cells to greater concentrations of serum or for longer incubation intervals, could have increased both von Willebrand factor and tissue factor. It is interesting, however, that under the same conditions endotoxin caused only a modest increase (33%) in cellular fibronectin whereas von Willebrand factor and tissue factor increased 2.6-fold and 18-fold, respectively.

A possible explanation for the current findings and our previous data demonstrating enhanced cytotoxicity3 and platelet-derived growth factor-stimulating effects⁴ of preeclamptic sera is the phenomenon of hemoconcentration in association with the reduced blood volume reported in this condition. This does not seem likely, as no statistical differences in hematocrit levels were detected between normal and preeclamptic groups, probably indicating the mild nature of the disorder in women in this study. The latter conclusion is supported by similar infant and placental weights, platelet counts, and serum creatinine concentrations in the two groups.

The presence of a factor(s) in the blood of preeclamptic women that can activate in vitro an endothelial function found in the clinical disorder in vivo supports the hypothesis that endothelial cell activation or injury in preeclampsia could be secondary effects of changes distant from the site of the perturbed endothelium. We propose that this factor(s) is derived directly or indirectly from the poorly perfused trophoblast characteristic of preeclampsia and that maternal

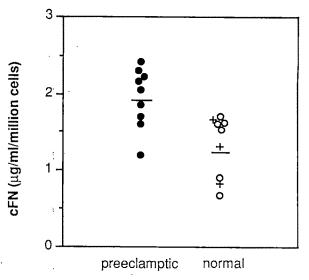


Fig. 1. Effects of predelivery pregnancy sera on human umbilical vein endothelial cell cellular fibronectin release in vitro. Human umbilical vein endothelial cells were exposed for 24 hours to media containing 10% predelivery pregnancy serum. Concentration of cellular fibronectin released into conditioned media was determined by ELISA. Serum samples from nine nulliparous preeclamptic patients (•), six nulliparous normal patients (0), and three parous normal patients (+) stimulated cellular fibronectin release as indicated. Horizontal bars, Mean cellular fibronectin concentrations for each patient group. Preeclamptic and normal patient groups differ significantly by Mann-Whitney and unpaired Student t tests (p = 0.01).

vascular endothelial cell injury or activation is a secondary, but essential, process in the pathogenesis of the preeclampsia syndrome.1

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Potential role of endothelin-1 in normal and hypertensive pregnancies

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Endothelins are the most potent naturally occurring vasoconstrictors yet discovered. Both normal and abnormal pregnancies are associated with significant changes in vascular smooth muscle; therefore the potential role of endothelin in pregnancy was investigated. Plasma immunoreactive endothelin-1 concentration was measured by radioimmunoassay in blood from women with normal pregnancy and preeclampsia and in cord blood from normal pregnancies. Endothelin-1 levels were elevated in pregnant women during labor when compared with levels in nonpregnant women and patients with normal pregnancies before labor. Preeclampsia in nonlaboring women before treatment was associated with higher endothelin values when compared with values in normal nonlaboring patients and women with preeclampsia after magnesium sulfate infusion. The umbilical venous concentration of endothelin was 10 times higher than normal pregnant levels and four times higher than levels in laboring patients. (AM J OBSTET GYNECOL 1991;165:1711-6.)

Key words: Endothelin, pregnancy, preeclampsia, labor, fetus

The role of the endothelium in modulating cardiovascular homeostasis via regulation of the vascular smooth muscle tone has been recognized recently. Endothelium regulates the reactivity of the smooth muscles through production of both vasodilators and vasoconstrictors. The endothelins are a group of vasoactive peptides first isolated from bovine aortic endothelial cells and are the most potent vasoconstrictors yet discovered. Three distinct human endothelin genes have been identified. Endothelin-1 is the only endothelin made by human endothelial cells. Endothelin-2 is produced in the kidney and endothelin-3 is mainly associated with neural tissue.

Endothelin appears to play a role in the pathogenesis of some forms of hypertension.⁴⁻⁶ Additionally, endothelin concentration is elevated in patients with acute myocardial infarction or vasculitis and in patients with uremia who are undergoing hemodialysis.⁷⁻⁹

Preeclampsia, a hypertensive disorder unique to pregnancy, is characterized by generalized vasoconstriction that is due, in part, to increased sensitivity of

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vascular smooth muscle to the effects of vasopressors. ¹⁰ This is in contrast to normal pregnancy where the vascular bed is less sensitive to vasoconstrictors. ¹⁰ Several lines of evidence implicate endothelial cell injury as a basic pathogenic mechanism in preeclampsia resulting in impaired synthesis of vasodilators and a possible increase in the production of vasoconstrictors. ¹¹ Preliminary studies suggested that endothelin concentrations are elevated in women with preeclampsia, compared with controls. ^{12, 18} Both normal and hypertensive pregnancies are associated with significant changes in vascular smooth muscle tone; therefore the potential role of endothelin in various pregnancy conditions and pregnancy complications was investigated in this study.

Material and methods

One hundred twenty-eight plasma samples were prospectively collected from volunteers presenting for care at the University of South Florida and the Tampa General Hospital. Eighteen samples were collected from nonpregnant women of reproductive age without any medical or gynecologic complications. Forty-seven samples were collected from women with normal pregnancies at several gestational age intervals (11 samples from 6 to 12 weeks, 19 samples from 16 to 20 weeks, 9 samples from 26 to 33 weeks, and 8 samples from 35 to 42 weeks). Sixteen samples were collected from nonlaboring patients with preeclampsia before (n = 10) or after (n = 6) the institution of magnesium sulfate infusion. Sixteen samples were collected from patients in labor (eight patients with normal pregnancies and eight with preeclampsia). The collection of blood from hospital-

Table I. Comparison of normal, hypertensive, and umbilical venous groups (mean \pm SD)

	Normal pregnancies, patients in labor (n = 8)	Umbilical venous group (n = 31)	Patients with preeclampsia (n = 24)	
Age (yr)	21.9 ± 3.4	^{21.8 ± 5.6}	21.5 ± 4.5	. ·
Gestational age at delivery (wk)	38.6 ± 4	39.1 ± 1.2	34.4 ± 4.6	•
Systolic pressure (mm Hg)	126.2 ± 14	122.2 ± 8.9	160 ± 22	
Diastolic pressure (mm Hg)	75 ± 5	72.6 ± 7.8	106 ± 13	
Hematocrit (%)	34.3 ± 3.7	36.01 ± 2.8	34.5 ± 5.25	, .
Platelets (10 ³ /mm ³)	196 ± 44	227 ± 72	189 ± 68	
Uric acid (mg/dl)	4.1 ± 0.26	. —	6.5 ± 1.3	*
Birth weight (gm)	3135.1 ± 893.4	3355 ± 446	2245 ± 953	
Vaginal delivery (no.)	4 (50%)	19 (61.3%)	16 (66.7%)	
Cesarean section (no.)	4 (50%)	12 (38.7%)	8 (33.3%)	,

ized women was done on admission to the labor and delivery suite with the patients in the supine position and before any medications and thus reflects the first stage of labor in laboring women. Thirty-one samples were collected from the umbilical vein immediately after delivery in normal term pregnancies, before and after umbilical cord clamping.

All patients gave written informed consent as approved by the University of South Florida's Institutional Review Board. Preeclampsia was defined according to the following criteria: (1) nulliparity, (2) blood pressure $\geq 140/90$ mm Hg measured on two occasions at least 6 hours apart, (3) proteinuria of ≥ 30 mg/dl (defined as +1) by dipstick on random catheterized specimen, (4) no known history of hypertension before pregnancy, and (5) resolution of hypertension and proteinuria in the postpartum period.

All volunteers with normal pregnancies denied a history of hypertension or other medical complications. Patients with a history of illicit drug use or other medications were not included. Blood pressure measurements were obtained from patients in the sitting position after 15 minutes of rest, with phase IV Korotkoff sound used for the diastolic blood pressure. Obstetric and demographic data were recorded from the medical records. Blood samples were collected from each patient in the sitting position. Plasma was prepared from blood collected into chilled tubes containing 5 mg/ml ethylenediaminetetraacetic acid and 500 U/ml aprotinin. The plasma was separated by centrifugation and the samples were stored at -80° C until assayed. On the day of the assay, 2 ml of thawed plasma was acidified. with an equal volume 0.1% trifluoroacetic acid and 2N hydrochloric acid (pH 3), extracted over a C18 column, and eluted with 60% acetonitrile in 0.1% trifluoroacetic acid. Recoveries of 90% for both radiolabeled and unlabeled samples were observed. These extracts were evaporated to dryness under nitrogen and then assayed in duplicate with a specific radioimmunoassay (Peninsula Laboratories, Inc., Belmont, Calif.). Interassay and intraassay coefficient of variation for endothelin-1 was 7%.

Endothelin concentrations were corrected for midpoint displacement of each curve. We did not find differences in values from samples processed before or after freezing. Cross-reactivity with endothelin-2 and endothelin-3 was 7%. The person performing the radioimmunoassays was blinded as to the clinical condition of the patient. All data were expressed as mean \pm SD and were analyzed by the SAS (Statistical Analysis Systems, Cary, N.C.) statistical package, with the general linear model procedure for unbalanced designs, Duncan's multiple range test, unpaired and paired Student t tests, and χ^2 tests where appropriate. A probability of p < 0.05 was considered significant.

Results

The endothelin-1 concentration in normal nonpregnant women of reproductive age (mean age 32.4 ± 4.7 years, range 30 to 39 years) was 1.8 ± 0.6 pg/ml. There was no significant association between the subject's age and endothelin level concentrations for either nonpregnant or pregnant women and there was no significant difference in mean age between patients with normal pregnancies (28 ± 5 years) and nonpregnant controls.

All patients in the antepartum group remained normotensive and were delivered of normal infants at term. In Table I obstetric and demographic data are presented for the normal labor, preeclampsia, and umbilical venous sample groups. Systolic and diastolic blood pressure, urinary protein concentration, and serum uric acid concentration were significantly higher (p < 0.001) in the patients with preeclampsia. Endothelin-1 concentration in the nonpregnant and antepartum groups is shown in Fig. 1.

We did not observe a difference in endothelin concentrations between pregnant and nonpregnant patients (by SAS General Linear Model procedure for unbalanced designs). A possible effect of gestational

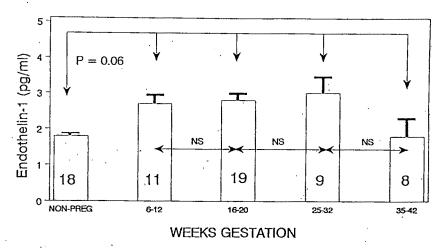


Fig. 1. Effect of pregnancy on peripheral venous plasma endothelin-1 levels (Duncan's multiple range test).

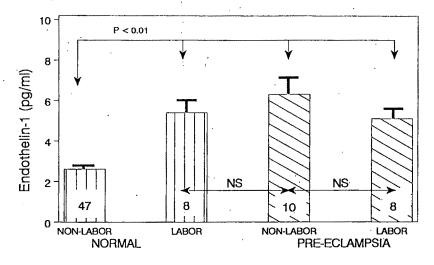


Fig. 2. Effect of labor on maternal venous plasma endothelin-1 in normal pregnancy and preeclampsia (Duncan's multiple range test).

age with increasing endothelin concentrations in the second and early third trimesters did not reach statistical significance (p = 0.06).

As noted in Fig. 2, levels of endothelin-1 were significantly higher in laboring women than in nonlaboring women and all patients with preeclampsia had concentrations higher than those in nonlaboring women with normal pregnancies. The increase noted in women with preeclampsia was similar to that associated with labor (SAS, General Linear Model procedure).

Women with preeclampsia (samples taken before magnesium sulfate infusion) had significantly elevated plasma endothelin concentrations when compared with samples obtained during magnesium sulfate infusion in nonpaired samples (p < 0.05) (Fig. 3). Similar results were found when paired samples were used (endothelin level before magnesium sulfate, $6.6 \pm 3.9 \text{ pg/ml}$; after magnesium sulfate, $4.75 \pm 2.9 \text{ pg/ml}$ (p < 0.02). Furthermore, endothelin was elevated in nonlaboring women with preeclampsia before treatment compared with patients with normal pregnancy (p < 0.001). Women with preeclampsia who labored had endothelin concentrations similar to those of laboring patients with normal pregnancies (Duncan's multiple range test) (Fig. 3). All patients with preeclampsia had hematocrits that were similar to those of patients with normal pregnancies.

In nonpaired samples the umbilical venous concentration of endothelin was 10 times higher than in normal pregnancies and four times higher than in laboring women (p < 0.001) (Fig. 4). Similar data were found in paired samples from mothers in labor and umbilical

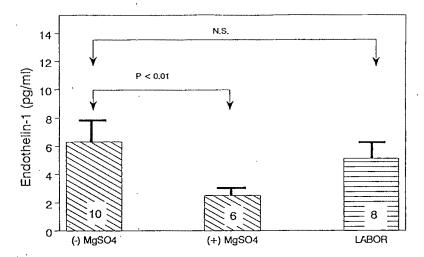


Fig. 3. Effect of magnesium sulfate and labor on maternal venous plasma endothelin-1 levels in women with preeclampsia (unpaired Student t test).

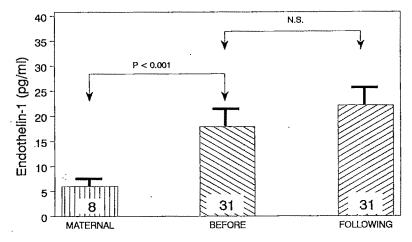


Fig. 4. Maternal venous and umbilical venous endothelin-1 levels before and after cord clamping (unpaired Student t test).

veins at delivery (maternal endothelin-1 4.0 ± 3.26 pg/ml, umbilical vein endothelin-1 17.6 ± 10.9 pg/ml). The concentration of endothelin was not different before or after cord clamping in paired samples (Fig. 4). Furthermore, no difference in umbilical venous samples from vaginal deliveries (n = 19) compared with cesarean sections (n = 12) was found.

Endothelin concentrations were not different in patients delivered by cesarean section and in those delivered vaginally.

No correlation between endothelin values (in the preeclampsia group) and level of maternal blood pressure or clinical severity was observed.

Comment

Endothelin-1 is the most active vasopressor substance yet discovered, with a potency 10 times that of angiotensin II.² Other properties of endothelin include in-

duction of uterine contractions, release of prostaglandins, and the release of endothelial-derived relaxing factors. 14-16 It is now believed that endothelin-1 binds to a specific membrane receptor leading to intracellular biochemical signals involving the activation of phospholipase C, with a release of inositol phosphates and diacylglycerol and mobilization of intracellular calcium. The resultant increase in intracellular calcium activates protein kinase C. 14, 17 Many substances, including thrombin, epinephrine, and the calcium ionophore A23187, cause the slow release of immunoreactive endothelin-1 from cultured endothelial cells. 2

In healthy humans plasma levels of endothelin-1 measured by radioimmunoassay have been estimated to range from 0.26 to 5 pg/ml. The concentration of endothelin-1 is likely to be much higher at the interface between the endothelium and smooth muscle. Therefore endothelin-1 is more likely to act as a local autacoid

rather than as a systemic regulating hormone.19 Paracrine effects of endothelin are very important, because many pharmacologic actions of endothelin-1 are not substantiated naturally by the systemic concentrations found, which are much lower than those required for the actions observed in vitro.18 This is particularly true because the increased concentrations of endothelin-1 are not necessarily related to the expected degree of vasoconstriction but are probably secondary effects of the simultaneous production of endothelial-relaxing factors and of vasodilatory prostaglandin release.18 It is also worth noting that the kidneys are 10 times more sensitive than other vascular regions to the vasoconstrictor effects of endothelin-1.20

The potent vasoconstrictor action of endothelin-1, together with elevated plasma levels found in myocardial infarction, vasculitis, and essential or pulmonary hypertension, contributes to the hypothesis that endothelin-1 may be involved in hypertensive states. 4-8 Its ability to stimulate the release of aldosterone and catecholamines from adrenal glands may contribute to hypertension, as would its ability to stimulate the release of renin.20, 21 In laboratory animals the sensitivity of renal artery segments to endothelin-1 was greater in rats with spontaneous hypertension than in normal animals.22

Normal pregnancy state is characterized by generalized refractoriness to vasopressor substances such as angiotensin II.10 On the contrary, pregnancy-induced. hypertension is characterized by widespread vasoconstriction.10 The dominant view is that the vasoconstriction results mainly or wholly from an abnormal sensitivity of vascular smooth muscle to the vasoconstrictive effect of pressor substances.

This study was concerned with endothelin-1 levels found in normal and preeclamptic pregnancies during labor, as well as in umbilical vein blood. Endothelin levels were elevated in women with preeclampsia without labor before any institution of treatment. This finding confirms that of Taylor et al.12 and Kamoi et al.13 Furthermore, we showed that magnesium infusion lowers the level of endothelin as compared with the preinfusion level. Surprisingly, no difference in endothelin values was found in laboring patients with preeclampsia compared with laboring patients with normal pregnancies.

We cannot explain at this time the high endothelin levels in normal pregnancies in the first stage of labor. It has recently been proposed, however, that endothelin-1 and oxytocin modulate calcium through independent receptors, and endothelin, like oxytocin, is an important modulator of uterine contractibility with possible implications in the physiologic characteristics of normal labor.28

The mechanism of endothelin elevation in preeclampsia is unknown, but there are a few possible ex-

planations. First, endothelin is basically a locally acting factor at the junction between endothelium and smooth muscle layer; therefore disruption and destruction of these anatomic boundaries can lead to a leak of endothelin from its local environment to the bloodstream with subsequently higher peripheral blood levels. Second, an abnormal production of endothelin by the affected endothelium might be a primary mechanism for the increase of endothelin locally and in the bloodstream. Third, increased production of endothelin from placental or fetal tissue in preeclampsia, or increased diffusion into the maternal circulation, might explain the elevated levels found in preeclampsia. Supporting the last hypothesis is evidence that the endothelin-1 receptor was identified in the membranes from a human placenta.²⁴ Endothelin is found in the amniotic fluid and it is shown to be synthesized not only by endothelial cells derived from human umbilical vein but also by amnion cells.25

Last, a defect of endothelin clearance could be a mechanism for the increased levels in preeclampsia. A different plasma volume of distribution in preeclampsia versus normal pregnancy would be an important factor. In our study the hematocrits did not differ significantly between the normotensive and preeclamptic groups. However, plasma albumin concentration may be a better indicator of plasma volume, independent of iron stores. Unfortunately, we did not test albumin concentrations in our patients.

Pharmacologic concentrations of endothelin-1 increase prostacyclin release from endothelial cells in vitro.15,26 This property may counterbalance its vasoconstrictor effects in vivo and serve as a negative feedback. Given the observation that the prostacyclin levels are reduced, in preeclampsia, then an abnormality of endothelin-prostacyclin interaction could exist.

We observed a lowering of endothelin values in patients during magnesium sulfate infusion. Recent in vitro studies have suggested that magnesium increases the prostacyclin production from endothelial cells, an observation that could not be supported in vivo as measured by systemic prostacyclin metabolite excretion in women who received magnesium sulfate infusion.27 However, an increase in renal prostacyclin production was observed in patients in preterm labor after magnesium sulfate infusion. Endothelin has a major action on the glomerulus and is synthesized by kidney cells, so that a change in the prostacyclin environment in the kidney could play some role in addition to the vasodilatory effect with subsequent release of the stretch stimulus of the vessel caused by magnesium sulfate. A recent study11 that also found increased levels of endothelin-1 in patients with preeclampsia failed to find a difference in the second-trimester levels of this peptide in women in whom preeclampsia eventually developed. These data support the hypothesis that endothelin elevation in preeclampsia is probably a secondary effect of another mechanism that initiates the change in endothelium that is basic to this disease.

The high concentration of endothelin-1 in the umbilical venous circulation in our study supports the previous report by Usuki et al. 25 and cannot be adequately explained at this time.24,26 Our data do not support a role in the contraction of umbilical vessels after delivery, because we did not find increased levels over a course of time (from before to after cord clamping). However, other investigators have suggested that endothelin-1 may contribute to fetal hemodynamic change, e.g., closure of the umbilical vessels occurring at delivery.28 These investigators collected cord blood after the clamping of the umbilical cord. As mentioned earlier, endothelin was found to be synthesized by endothelial cells derived from umbilical cords and amnion cells,25 suggesting that endothelin might play a significant role in the regulation of uteroplacental circulation. Endothelin was reported to contribute to the closure of the ductus arteriosus in lambs, emphasizing the importance of this peptide in gestation.29 Further study is needed to clarify this issue.

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Endothelin-1-induced vasoconstriction is not mediated by thromboxane release and action in the human fetal-placental circulation

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The vasoconstrictor peptide endothelin-1 (8 \times 10⁻¹⁰ to 1 \times 10⁻⁸ mol/L) significantly increased fetal-placental perfusion pressure in vitro in a cumulative manner from 30 \pm 2 to 123 \pm 25 mm Hg (mean \pm SEM, n=5, p<0.0005, analysis of variance). Accompanying this vasoconstriction was a corresponding reduction in fetal-placental perfusate flow rate. Measurement of thromboxane B₂ and 6-keto-prostaglandin F_{1a} in the fetal-placental perfusate revealed a significant reduction in their release (p<0.0096 and p<0.0004, analysis of variance, respectively) when corrected for flow rate. Neither the thromboxane synthesis inhibitor dazoxiben (10⁻⁶ mol/L) nor the thromboxane receptor antagonist SQ29548 (10⁻⁶ mol/L) was able to block the vasoconstrictor actions of endothelin-1. Therefore endothelin-1—induced vasoconstriction in the human fetal-placental circulation does not appear to be mediated by thromboxane release or action. The stimulus to eicosanoid release in the fetal-placental circulation may be hydrodynamic, i.e., flow or shear stress. (AM J OBSTET GYNECOL 1991;165:1717-22.)

Key words: Endothelin, thromboxane, placenta, vasoconstriction

The endothelins are a family of three distinct 21amino-acid peptides, endothelin-1, endothelin-2, and endothelin-3, which are coded for by three separate genes in the human rat and porcine genomes.1 Recently two distinct receptors for the endothelins have been cloned.2,3 The endothelins were first described as vasoconstrictor peptides produced by endothelial cells, although recently other cellular sites of synthesis and actions as diverse as oxytocic activity on the uterus4 and stimulation of peptide hormone release⁵ have been reported. The vasoconstrictor action of endothelin is characteristically long-acting (45 to 60 minutes) and is more pronounced on veins than on arteries.⁶ There is also evidence that threshold concentrations of endothelin increase sensitivity to other vasoconstrictors such as norepinephrine and serotonin.7.8 The action of endothelin is thought to be mediated by voltage-sensitive or, non-voltage-sensitive plasma membrane calcium channels.4

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Reprint requests: L. Myatt, PhD, Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, 231 Bethesda Ave., Cincinnati, OH 45267-0526. 6/6/32384 There are reports that the contractile effect of endothelin-1 is mediated by thromboxane release and action. In isolated guinea pig airways⁹ and in rat vascular smooth muscle cells,¹⁰ endothelin stimulated thromboxane biosynthesis, whereas specific thromboxane receptor blockers⁹ or inhibitors of thromboxane synthesis¹⁰ attenuated the effects of endothelin-1. However, in human lung¹¹ the contractile effect of endothelin-1 does not appear to be due to release of prostaglandins or thromboxane A₂.

In the perfused human placenta, endothelin-1 has been shown to have a potent vasoconstrictive action¹² and to cause an apparent release in thromboxane during the contraction. Interestingly, the same workers¹³ previously had shown that the vasoconstrictive effect of bradykinin on the vessels of the perfused human cotyledon was associated with thromboxane release and action and could be partially attenuated with the thromboxane receptor antagonist SQ29548.

During vasoconstriction of the human fetal-placental circulation in vitro, a reduction in perfusate flow rate may account for the apparent increase in concentration of autocoids released into the perfusate. Therefore we have measured both thromboxane and prostacyclin release by the human fetal-placental circulation during endothelin-1—induced vasoconstriction in relation to perfusate flow rate. Further, we have used inhibitors of thromboxane synthesis and action to determine the role of thromboxane in endothelin-1—induced vasoconstriction.

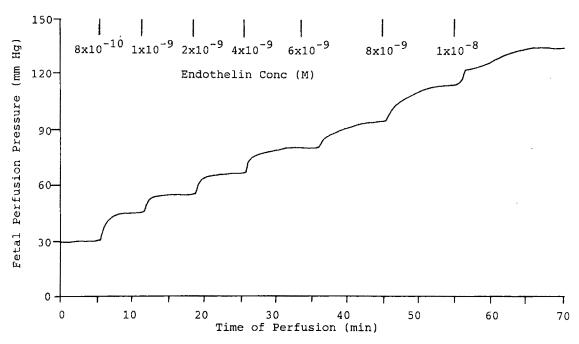


Fig. 1. Representative experiment of effect of endothelin-1 (8 \times 10⁻¹⁰ to 3.18 \times 10⁻⁸ mol/L cumulative concentration) on fetal-placental perfusion pressure.

Material and methods

Placentas were collected immediately after normal vaginal delivery or elective cesarean section, under a protocol approved by the institutional review board, and were transported to the perfusion laboratory in the labor and delivery area. A suitable third- or fourthorder chorionic artery and corresponding vein of an intact cotyledon were cannulated, as we have previously described,14 at a point immediately before passage of the vessels through the chorionic plate. The maternal intervillous space was cannulated with two butterfly needles inserted through remnants of the spiral arteries in the basal plate. The effluent from the intervillous space was collected by gravity into a Plexiglas cone over which the cotyledon was placed, maternal surface downward. Perfusion medium, except where stated, was tissue culture medium 199 containing 5% polyvinylpyrrolidone 40 and 0.1% bovine serum albumin as oncotic agents and with 20 IU/ml heparin and 48 μg/ml gentamicin added. Medium was pH 7.4 with bicarbonate and gassed with 95% oxygen and 5% carbon dioxide at 37° C. Flow rates on the perfusion pumps were 4 (fetal) and 10 (maternal) ml/min except where stated. Lateral pressure was measured in fetal and maternal inflow lines adjacent to the point of cannulation. Data on pressure, inflow and outflow Po2, and pH were sampled every second and stored on an IBM PS2 system with Asystant Plus software (Asyst Technologies, Rochester, N.Y.).

The effects of endothelin-1 on perfusion pressure and eicosanoid release in the fetal-placental circulation was studied. After an equilibration period of at least 30 minutes, endothelin-1 (8 \times 10⁻¹⁰ mol/L) was infused as a 2 ml bolus to the fetal-placental circulation. When perfusion pressure had increased, the next concentration of endothelin-1 was infused (8 to 10-10 to 1×10^{-8} mol/L) and cumulative increases in perfusion pressure recorded. Both maternal and fetal perfusates were collected in fractions corresponding to a 5-minute duration of flow. The volume collected in each fraction was then measured to calculate outflow from each circulation before the perfusate fractions were stored at -20° C for subsequent assay of thromboxane (TxB₂) B₂ and 6-keto-prostaglandin (PGF₁₀) F₁₀, the stable hydrolysis products of thromboxane A2 and prostacyclin, respectively, with radioimmunoassays we have previously described.15

To determine if thromboxane release and action were mediators of endothelin-1—induced vasoconstriction, the effects of a thromboxane synthetase inhibitor and a thromboxane receptor antagonist on endothelin-1 action were studied. The vasoconstrictor response to endothelin-1 (3 \times 10⁻⁸ mol/L) was measured before and after addition of the thromboxane synthetase inhibitor dazoxiben (10⁻⁶ mol/L) to both maternal and fetal perfusate. A 30-minute period of equilibration with dazoxiben was allowed before the second infusion of endothelin-1. Thromboxane receptor antagonist studies were conducted as follows: After a 30-minute equilibration period, vasoconstrictor responses to bolus infusion of the thromboxane mimetic U46619 (2.3 \times 10⁻⁷ mol/L) and endothelin-1 (3 \times 10⁻⁸

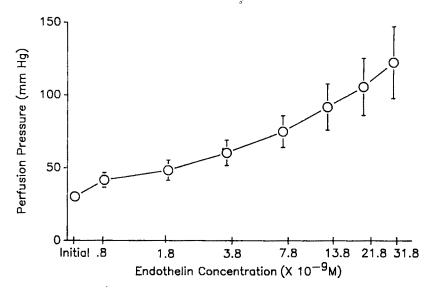


Fig. 2. Effect of endothelin-1 on fetal-placental perfusion pressure. Cumulative perfusion pressure on infusion of increasing concentrations (8×10^{-10} to 1×10^{-8} mol/L [8×10^{-10} to 3.18×10^{-8} mol/L cumulative]) of endothelin-1 on fetal-placental circulation. Mean \pm SEM, n = 5. One way analysis of variance showed significant increase in perfusion pressure with increasing concentrations of endothelin-1 (p < 0.0005).

mol/L) were recorded. After reequilibration of perfusion pressure after addition of this endothelin-1, both fetal and maternal perfusion media were changed to media containing SQ29548 (10⁻⁶ mol/L) and perfusion continued. After a 30-minute equilibration period with SQ29548, the responses to infusion of U46619 and endothelin-1 were again determined. All experiments were repeated in at least three separate placentas.

The significance of changes in perfusion pressure and eicosanoid synthesis on addition of increasing concentrations of endothelin-1 was determined by analysis of variance, and the effects of thromboxane antagonists were determined by paired t tests. U46619 was kept as a 10^{-3} mol/L stock in ethanol at -20° C and endothelin-1 was kept at 10^{-5} mol/L in 0.5 mol/L acetic acid at -70° C. Dazoxiben was kept at 10^{-3} mol/L in saline solution at 4° C and SQ29548 at 10^{-3} mol/L in ethanol at 4° C. Appropriate dilutions were made before each use.

Results

Endothelin-1 (8 × 10⁻¹⁰ to 1 × 10⁻⁸ mol/L [8 × 10^{-10} to 3.18×10^{-8} mol/L cumulative concentration]) gave a significant cumulative increase in fetal-placental perfusion pressure (p < 0.0005, analysis of variance from 30 ± 2 to 123 ± 25 mm Hg (Figs. 1 and 2). As shown in Fig. 1, the increase in perfusion pressure on addition of endothelin-1 was sustained (45 to 60 minutes) such that only cumulative increases could be measured. Accompanying the increase in perfusion pressure was a decrease in fetal venous perfusate outflow from an initial measured venous return of 3.1 ± 0.1

ml/min to 0.19 ± 0.08 ml/min (Fig. 3). Although the fetal perfusion pump flow rate was nominally set at 4 ml/min, fetal venous outflow was measured to accurately quantify fetal flow rate. The resting flow rate of 3.1 ml/min probably results from some loss of perfusate by transfer to the maternal circuit. Interestingly, there was a slight increase in flow rate, although not significant, at the lower concentration of endothelin-1.

Direct measurement of TxB_2 and 6-keto-PGF_{1 α} concentrations in the fetal venous perfusate showed an apparent increase in TxB_2 concentrations in the perfusate at the higher concentrations of endothelin-1 (data not shown) but with no apparent change in 6-keto-PGF_{1 α} concentrations. However, when corrections for the reduction in flow rate during endothelin-1-induced vasoconstriction were made (Fig. 4), there was an obvious decrease in the release of both TxB_2 from 987 ± 182 to 56 ± 10 pg/min (p < 0.0096, analysis of variance) and 6-keto-PGF_{1 α} from 332 ± 74 to 33 ± 11 pg/min (p < 0.0004, analysis of variance).

Addition of the thromboxane synthesis inhibitor dazoxiben (10^{-6} mol/L) to perfusate did not alter the vasoconstrictor response to endothelin-1 (3×10^{-8} mol/L). The vasoconstrictor responses to endothelin-1 before and after dazoxiben were 18.0 ± 7.4 and 25.1 ± 13.6 mm Hg, respectively (mean \pm SE, n = 5). However, addition of the thromboxane receptor antagonist SQ29548 (10^{-6} mol/L) significantly attenuated the vasoconstrictor response to U46619 (2.3×10^{-7} mol/L) in this preparation (Fig. 5 and Table I) but had no effect on the vasoconstrictor response to endothelin- $1 (3 \times 10^{-8}$ mol/L). The slightly greater and more vari-

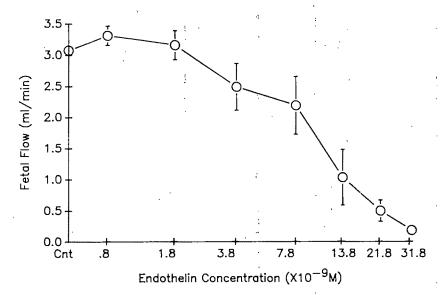


Fig. 3. Effect of endothelin-1 on fetal-placental perfusate flow rate. Fetal-placental flow rate was determined by measuring fetal venous effluent volume during endothelin-1-induced vasoconstriction. Mean \pm SEM, n=5.

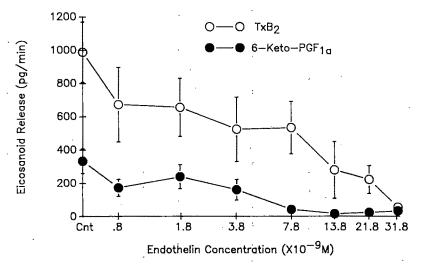


Fig. 4. TxB_2 and 6-keto-PGF_{1 α} release during endothelin-1 induced vasoconstriction. TxB_2 and 6-keto-PGF_{1 α} concentrations were measured by radioimmunoassay in fetal venous perfusate fractions collected during endothelin-1-induced vasoconstriction. Eicosanoid release was corrected for flow rate and expressed as picograms per minute. Mean \pm SEM, n=5. One-way analysis of variance showed significant reduction in TxB_2 release (p<0.0096) and 6-keto-PGF_{1 α} release (p<0.0004) with endothelin-1-induced vasoconstriction.

able response to endothelin-1 after either dazoxiben or SQ29548 may have been due to potentiation of the vasoconstrictor effect by the first endothelin-1 injection and by the difficulty in regaining baseline pressure after endothelin-1 injection that gave a very long-lasting effect (45 to 60 minutes).

Comment

In agreement with a previous report, ¹² we found that endothelin-1 was a potent vasoconstrictor of the human

fetal-placental circulation in vitro. The response to endothelin-1 is of sustained duration, purportedly acting by voltage-dependent or voltage-independent calcium channels; therefore only cumulative changes in perfusion pressure could be recorded. There was a reduction in perfusate flow rate (measured as fetal outflow) through the fetal circulation accompanying this vasoconstriction. This reduction in flow rate led to the apparent increase in thromboxane concentrations measured directly in perfusate at the higher endothelin

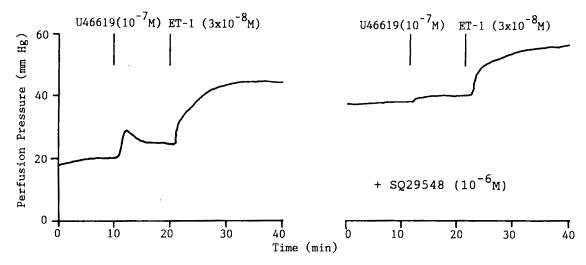


Fig. 5. Representative experiment of effect of SQ29548 (10⁻⁶ mol/L) on vasoconstrictor responses to U46619 and endothelin-1. Vasoconstrictor response to U46619 (2.3 × 10⁻⁷ mol/L) and endothelin-1 (3 \times 10⁻⁸ mol/L) is shown in same placenta before and during perfusion with thromboxane receptor antagonist SQ29548 (10⁻⁶ mol/L).

concentrations and also presumably in the previous report by Wilkes et al.12 Because endothelin-1 is a more potent constrictor of veins than arteries,6 it is possible that the effect of endothelin-1 is on the venous side of the cotyledonary vascular tree and that the reduction in fetal outflow relative to inflow may arise in part from bulk flow of water from the fetal to the maternal circulation as a result of the hydrostatic pressure difference resulting from vasoconstriction in the fetal circulation. We do not feel that the loss of fetal outflow was due to rupture of the fetal vasculature and a direct leak to the maternal circulation, as when this occurs, very erratic variations or large falls in perfusion pressure are seen. Correction for perfusate flow rate through the fetal-placental circulation revealed a reduction in both thromboxane and prostacyclin metabolite release per minute during endothelin-1-induced vasoconstriction. We have previously shown that increasing perfusate flow rate through the fetal-placental circulation led to increased release of both thromboxane and prostacyclin.16 Therefore, hydrodynamic forces (flow or shear stress) through the fetal-placental vessels may be the stimulus to eicosanoid release.

In some studies of different vasculatures, endothelin action has been shown to be mediated by thromboxane release and action by specific receptors,9,10 although other studies have demonstrated effects independent of thromboxane.11 We have clearly shown that inhibition of thromboxane synthesis by dazoxiben did not affect endothelin-1 action in the fetal-placental vasculature. Similarly, SQ29548, which effectively blocks thromboxane action by its receptor, was unable to prevent endothelin-1-induced vasoconstriction in the hu-

Table I. Perfusion pressure with and without addition of SO29548

	Perfusion pressure (mm Hg)		
	$U46619$ (2.3 × 10^{-7} mol/L)	Endothelin-1 (3 × 10 ⁻⁸ mol/L)	
Control With SQ29548 (10 ⁻⁶ mol/L)	20.6 ± 1.3 2.6 ± 1.1*	59.4 ± 21.6 55.6 ± 14.2	

Mean \pm SEM, n = 4.

*Significantly different from control (p < 0.00005, paired

man fetal-placental circulation. Therefore the action of endothelin-1 appears to be direct, probably mediated by calcium channels and not by release and action of thromboxane.

We are grateful to the labor and delivery staff of University Hospital for their assistance in providing placental tissues.

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An endothelial cell model for the investigation of the molecular regulation of fetal vascular tone

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Human umbilical vessels are not innervated, and hence regulation of tone in these vessels must come from locally derived vasoactive substances such as prostacyclin. To evaluate the regulation of prostacyclin production by human umbilical vein endothelial cells, we incubated confluent cultures of these cells with various concentrations of inflammatory mediators (endotoxin, interleukin-1 β , and tumor necrosis factor), protein kinase C agonists (phorbol 12-myristate 13-acetate, phorbol 12,13-dibutyrate), and calcium ionophores (A-23187 and ionomycin). Human umbilical vein endothelial cells were prepared from term pregnancies, and confluent cultures were incubated with test substances for 16 hours. Prostacyclin was measured as its metabolite 6-keto-prostaglandin $F_{1\alpha}$ by radioimmunoassay. Concentration-related increases in 6-keto-prostaglandin $F_{1\alpha}$ production were observed in response to endotoxin, cytokines, phorbol esters, and calcium ionophores. We conclude that human umbilical vein endothelial cell prostacyclin production is influenced by several intracellular messengers and that human umbilical vein endothelial cells may provide a useful in vitro model for investigating the physiology and pathophysiology of umbilical vessel vascular tone. (AM J OBSTET GYNECOL 1991;165:1723-6.)

Key words: Endothelium, prostacyclin, cytokine, calcium, protein kinase C

Because human umbilical vessels are not innervated, regulation of vascular tone in the umbilical cord must be dependent on local vasoactive substances, presumably derived from and produced by endothelium. Products of arachidonic acid metabolism through the cyclooxygenase pathway may be important in controlling the tone of umbilical vessels.^{1, 2} Prostacyclin, a potent vasodilator, is the primary cyclooxygenase product of human umbilical vein endothelium and may be of importance physiologically and pathophysiologically in the regulation of fetoplacental hemostasis and blood flow.^{3,5}

The purpose of this study was to determine the regulation of prostacyclin production (as measured by its stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ [6-keto-PGF_{1\alpha}]) by primary cultures of normal human umbilical vein endothelial cells in culture. We chose to evaluate the effects of inflammatory mediators, because their release during infection in the perinatal period may contribute significantly to subsequent neonatal mortality and morbidity. ^{6, 7} Additionally, we have evaluated the effects of protein kinase C modifiers and calcium ionophores on 6-keto-PGF_{1\alpha} production, because these intracellular signaling systems have significant effects

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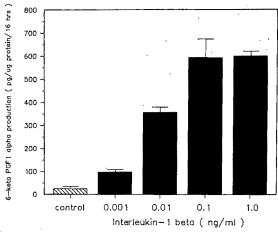
on arachidonic acid metabolism in other gestational cells and tissues.⁸⁻¹² An understanding of the regulation of arachidonic acid metabolism by primary cultures of human umbilical vein endothelial cells could provide significant insight into the determinants of normal and abnormal fetal umbilical vascular tone and blood flow.

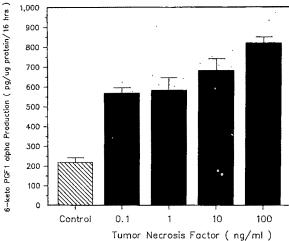
Material and methods

Material. M199 was obtained from Gibco, Long Island, NY; Dulbecco's modified Eagle's medium, Ham's F12/Dulbecco's modified Eagle's medium (1:1), and fetal calf serum were purchased from Irvine Scientific, Santa Ana, Calif. Penicillin (10,000 U/ml), streptomycin (10,000 μg/ml), and amphotericin B (25 μg/ml) solution was purchased from Gibco, Grand Island, N.Y. Cell culture plates were from Costar, Cambridge, Mass. Phorbol 12-myristate 13-acetate, phorbol 12,13-dibutyrate, 4α -phorbol didecanoate, A23187, ionomycin, and bacterial endotoxin from *Escherichia coli* were purchased from Sigma Chemical Company, St. Louis. Interleukin-1β (IL-1β) and tumor necrosis factor were purchased from R and D Systems, Minneapolis, Minn.

Primary human umbilical vein endothelial cells. Endothelial cells were obtained from the umbilical vein of normal term human pregnancies by the method of Jaffe¹⁵ and grown in M199 culture medium with 20% normal pooled heat-inactivated human serum and antibiotics, as we have previously described. ¹⁴ Confluent monolayers of human umbilical vein endothelial cells in 24-well culture plates were used for all the experiments described.

Experimental conditions. Dilutions of each bioactive





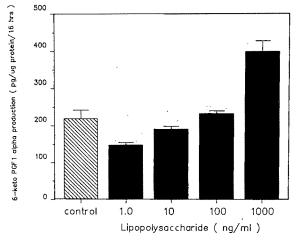


Fig. 1. Effect of inflammatory mediators on 6-keto-PGF_{ta} production (mean \pm SEM, N=4) by human umbilical vein endothelial cells. *Top panel*, IL-1 β (p < 0.01, control vs all concentrations). *Middle panel*, Tumor necrosis factor (p < 0.01, control vs all concentrations). *Bottom panel*, Lipopolysaccharide (p < 0.01, control vs 1000 ng/ml).

substance tested were made in experimental medium (Dulbecco's modified Eagle's medium and Ham's F12/Dulbecco's modified Eagle's medium [1:1] with 10% fetal calf serum and antibiotics) and then applied to the cells after the culture medium was aspirated.

Each condition was tested in quadruplicate, and in each experiment there were control cells (experimental medium only) and cells that underwent thrombin (international units per well) stimulation. Only plates of endothelial cells that demonstrated an adequate response to thrombin (at least two times control 6-keto-PGF_{1a} production) were considered valid and are represented in the results.

6-Keto-PGF_{1α} **production.** Cells were incubated with or without test substances for 16 hours, and media were collected and stored frozen until assayed for 6-keto-PGF_{1α} by radioimmunoassay, with an antiserum obtained from Advanced Magnetics, Cambridge, Mass. Total cell protein was determined by the method of Lowry et al. Results are expressed as picograms 6-keto-PGF_{1α} production per microgram protein per 16 hours. Statistical differences were assessed by the Student t test.

Results

Both IL-1 β and tumor necrosis factor induced significant concentration-dependent increases in 6-keto-PGF_{1 α} production by human umbilical vein endothelial cells (Fig. 1). Notably, IL-1 β was a particularly potent stimulator of 6-keto-PGF_{1 α} production, inducing a four-teenfold increase at just 0.01 ng/ml. Conversely, lipopolysaccharide stimulated 6-keto-PGF_{1 α} production only at the highest concentration tested (1000 ng/ml).

Both of the active phorbol esters phorbol 12-myristate 13-acetate and phorbol 12,13-dibutyrate stimulated significant increases in 6-keto-PGF_{1 α} production by human umbilical vein endothelial cells (Fig. 2). The inactive phorbol ester 4 α -phorbol didecanoate, however, had no effect on prostacyclin production.

Treatment with ionomycin and A23187 caused concentration-dependent increases in 6-keto-PGF_{1 α} production by human umbilical vein endothelial cells (Fig. 3). Relatively high concentrations of A23187 were required to stimulate significantly the production of prostacyclin, perhaps reflecting the relatively nonspecific action of this ionophore. Ionomycin was another potent stimulator of 6-keto-PGF_{1 α} production.

Comment

Our results indicate that prostacyclin production by human umbilical vein endothelial cells can be stimulated by a wide variety of agonists by means of different signal transduction mechanisms. Phorbol esters have been found to stimulate *de novo* synthesis of cyclooxygenase, ¹⁶ and our data support the finding that phorbol esters stimulate prostacyclin production in human umbilical vein endothelial cells. However, the exact signaling linkage between protein kinase C activation and arachidonic acid metabolism remains unclear. Increases in intracellular calcium mediated by the calcium ionophores A23187 and ionomycin also stimulate prostacyclin production. This action is presumed to be the

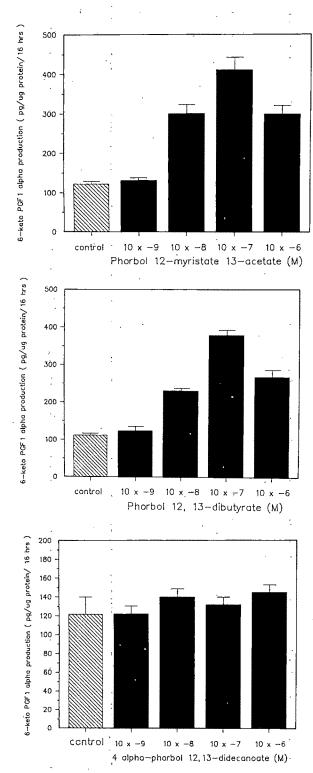
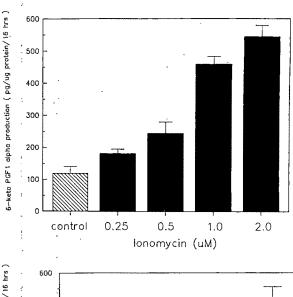


Fig. 2. Effect of phorbol esters on 6-keto-PGF_{1α} production (mean \pm SEM, N=4) by human umbilical vein endothelial cells. Top panel, Phorbol 12-myristate 13-acetate (p < 0.01, control vs 10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/L). Middle panel, Phorbol 12,13-dibutyrate (p < 0.01, control vs 10^{-8} , 10^{-7} , and 10^{-6} mol/L). Bottom panel, 4α -Phorbol didecanoate (p > 0.1, control vs all concentrations).

result of intracellular calcium activation of phospholipases, thus increasing available substrate for the cyclooxygenase enzyme. Inflammatory cytokines also were found to be potent stimulators of prostacyclin



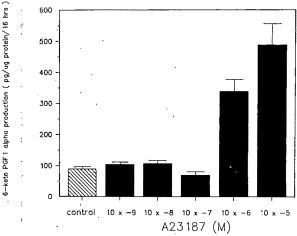


Fig 3. Effect of calcium ionophores on 6-keto-PGF_{ac} production (mean \pm SEM, N=4) by human umbilical vein endothelial cells. Upper panel, Ionomycin (p < 0.05, control vs all concentrations). Lower panel, A23187 (p < 0.05, control vs 10^{-7} and 10^{-6} mol/L).

production, and the cyclooxygenase gene has been reported to be induced in endothelial cells by IL-1B.17 However, the signaling mechanisms linking the IL-1 receptor to specific gene activation is not completely known but is thought to be dependent on activation of phospholipases.18

Regardless of mechanism, IL-1B appears to be a particularly potent stimulator of prostacyclin production, because concentrations as low as 0.01 ng/ml result in significantly elevated prostacyclin production. Interestingly, endotoxin has only a modest effect even at relatively high concentrations (1000 ng/ml). These data suggest that increased concentrations of prostacyclin resulting from IL-1\beta stimulation may play a role in the hypotension that is characteristic of gram-negative bacterial neonatal sepsis.6,7 This finding is consistent with previous reports that an overly exuberant host response to bacterial invasion, as reflected by production of inflanimatory cytokines, is responsible for several of the serious sequelae characteristic of sepsis in adult patients.^{19,20} Our findings confirm that such a mechanism in fetal tissue is possible. However, it is uncertain whether this may account for the neonatal signs and symptoms of sepsis or provide further rationale for the development of interventional therapies designed to interrupt the cytokine-mediated inflammatory cascade.^{21, 22}

It is readily evident that endothelial cells obtained from the umbilical cords of term pregnancies provide a useful model for the understanding of signal transduction mechanisms in general, but human umbilical vein endothelial cells may not be entirely appropriate to study adult endothelial cell properties and functions. For example, we have previously shown that human umbilical vein endothelial cells are not useful as a model to evaluate the effects of antiphospholipid antibodies on prostaglandin production.14 However, we believe that our data indicate that primary cultures of human umbilical vein endothelial cells may be a useful model for the understanding of the molecular events that regulate fetal vascular tone. Although we limited these studies to the regulation of endothelial cell prostacyclin production, the regulation of other vasoactive substances (e.g., endothelins) can be studied in a similar fashion.

We thank the staff of our labor and delivery unit for assistance with obtaining umbilical cords. We are grateful to Mary Ann Isenhart for expert editorial assistance and Samuel S. Edwin for artwork.

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In utero diagnosis of congenital varicella zoster virus infection by chorionic villus sampling and polymerase chain reaction

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Detroit, Michigan, Münster, Germany, and Bethesda, Maryland

Varicella zoster virus infection acquired in pregnancy is reported to cause fetal damage in 5% to 10% of cases. We used polymerase chain reaction to attempt molecular diagnosis of fetoplacental varicella zoster virus infection in two patients. Tissue obtained by chorionic villus sampling in the second trimester was analyzed by polymerase chain reaction with a varicella zoster virus—specific primer, ORF-63, and was found to be positive in both patients. Viral cultures were negative. One patient elected pregnancy termination at 23 weeks. Southern blot hybridization of neonatal brain tissue for varicella zoster virus was negative. The second patient carried the pregnancy to term and was delivered of a normal infant. Varicella zoster virus immunoglobulin M and viral cultures were negative. The presence of viral deoxyribonucleic acid sequences in placental tissue does not correlate with fetal disease. (AM J OBSTET GYNECOL 1991;165:1727-30.)

Key words: Chorionic villus sampling, polymerase chain reaction, varicella zoster virus

Varicella zoster virus infection acquired in pregnancy is reported to cause fetal damage in 5% to 10% of cases. ^{1, 2} Antenatal diagnosis of the fetal varicella syndrome has been achieved by ultrasonographic detection of fetal anomalies and by cordocentesis for the detection of fetal immune globulin production. ^{3, 4} Here we report the use of the polymerase chain reaction to attempt molecular diagnosis of fetoplacental varicella zoster virus infection.

Case reports

Case 1. Chickenpox lesions developed in a 17-year-old primigravid woman with onset at 4 to 5 weeks' gestational age. Lesions lasted 1 week. She desired evaluation for possible fetal infection and was seen in consultation at 19 weeks. Ultrasonographic examination showed no fetal defects. Viral cultures from chorionic villus sampling and amniocentesis were negative. However, polymerase chain reaction was positive. The patient elected pregnancy termination at 23 weeks' gestation. Cordocentesis was attempted but was not successful. The fetus was delivered after urea amnioinfusion and prostaglandin induction of labor. Autopsy of the fetus was unremarkable. Cord blood varicella zoster virus immunoglobulin M was unobtainable. Southern blot analysis of fetal brain tissue dem-

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onstrated no varicella zoster virus-specific genomic material.

Case 2. In a 30-year-old woman, gravida 2, para 2, chickenpox developed at 8 weeks' gestational age. Lesions lasted 1 to 2 weeks. She desired evaluation for possible fetal infection. After consultation at 20 weeks, she underwent transabdominal chorionic villus sampling and amniocentesis. Results of ultrasonographic evaluation were normal. Viral cultures on chorionic villus sampling and amniotic fluid material were negative. Polymerase chain reaction was positive for the presence of viral genome in trophoblast. She declined cordocentesis. Serial ultrasonographic examinations gave negative results. The patient was delivered of a healthy infant at term. Cord blood varicella zoster virus immunoglobulin M was negative and placental viral cultures were negative.

Material and methods

Deoxyribonucleic acid (DNA) extraction. The protocol of Miller et al.,5 was modified to extract deoxyribonucleic acid (DNA) from chorionic villus sampling and concurrently from control whole human fibroblasts (Whittaker MA Bioproducts, Walkersville, Md.). The tissue samples or whole human fibroblasts were placed in 2.25 ml of cold nuclear lysis buffer (0.5 mol/L Tris hydrochloride, pH 8; 20 mmol/L ethylenediaminetetraacetic acid, pH 8; and 10 mmol/L sodium chloride and homogenized with a Tissumizer homogenizer (Tekmar Co., Cincinnati). The cell lysates were digested at 50° C for 45 minutes with 0.125 ml of 20% sodium dodecyl sulfate and 0.125 ml protease K (20 mg/ml stock in distilled water). A total of 0.625 ml of saturated sodium chloride (6 mol/L) was added, and the lysate was shaken vigorously. The mixture was centrifuged

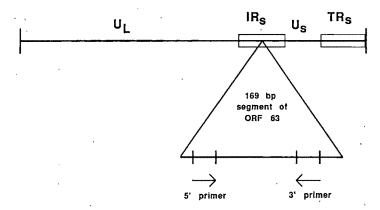


Fig. 1. Schematic map of varicella zoster virus genome showing location of amplified segment of open reading frame (ORF) 63 relative to unique long (U_L) , inverted repeat short (IR_S) , unique short (U_S) , and terminal repeat short (TR_S) elements. Also shown are locations of primer pairs used in polymerase chain reaction.

for 20 minutes at 2000 rpm, and the supernatant was transferred to another tube. DNA was precipitated by the addition of 5 ml of 100% ethanol and transferred to another tube with a plastic pipette. The DNA was washed once with 70% ethanol, dried in a Speed Vac (Savant Instruments, Inc., Hicksville, N.Y.), and suspended in Tris hydrochloride—ethylenediaminetetraacetic acid, pH 8; the concentration was determined with a DU-40 spectrophotometer by absorption at 260 nm (Beckman Instruments, Inc., Fullerton, Calif.).

Polymerase chain reaction. Oligonucleotide primers (18 mer) were chosen from published sequences of varicella zoster virus DNA and were synthesized with a Gene Assembler Plus DNA synthesizer (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.).6, 7 The primers (upstream 5' CGGTTGGATGTTAACGGA 3'; downstream 5' CCCGACGACTGTGGAATA 3') were designed to amplify a 169 bp segment of the gene encoding open reading frame 63. Fig. 1 shows a diagramatic representation of primer location within the varicella zoster virus genome. This schematic map shows the location of the amplified segment of open reading frame 63 relative to the unique long, inverted repeat short, unique short, and terminal repeat short elements. Also shown are the locations of the primer pairs used in the polymerase chain reaction. The 5' primer includes bases 110,603 through 110,720; the 3' primer includes bases 110,654 through 110,771.

Polymerase chain reaction was performed with a commercial kit (GeneAmp, Perkin Elmer Cetus, Norwalk, Conn.). All reactions were performed in 100 µl volumes and consisted of 0.5 µg of chorionic villus sampling tissue DNA or whole human fibroblasts DNA or 1 ng of purified varicella zoster virus virion DNA, 1 µmol of each primer, 200 µmol of each deoxynucleotide triphosphate (deoxyadenosine triphosphate,

deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythynidine triphosphate), 2.5 units of *Thermus aquaticus* DNA polymerase, 0.010 mol/L Tris—hydrochloric acid, 0.050 mol/L potassium chloride, and 0.015 mol/L magnesium chloride. The reaction mixtures were overlayed with 50 µl of mineral oil and placed in an automated thermal cycler (Perkins Elmer Cetus). The reaction conditions consisted of denaturation at 94° C for 1 minute, primer annealing at 50° C for 1 minute, and primer extension 72° C for 1 minute.

Analysis of polymerase chain reaction products. One microliter of 10× buffer (25% glycerol, 2.25 mmol/L ethylenediaminetetraacetic acid, 0.4 mmol/L Tris-hydrochloric acid, and 0.5% sodium dodecyl sulfate) was added to 10 µl aliquots of the polymerase chain reaction mixtures, and gel electrophoresis was performed with 1% agarose gel in buffer at a current of 100 mA. The gel was denatured for ½ hour in 1 mol/L sodium chloride and 0.5 mol/L sodium hydroxide and then neutralized for 1/2 hour in 0.5 mol/L Tris-hydrochloric acid, pH 7.5, and 1.5 mol/L sodium chloride. The DNA from the gel was transferred to a Nytran membrane (Schleicher and Schuell, Keene, N.H.) with a Posiblot transfer system (Stratagene, La Jolla, Calif.). The EcoRI-E restriction fragment of varicella zoster virus DNAs was used as the hybridization probe. It was radiolabeled with deoxycytidine triphosphate-5'(α-phosphorus 32), triethylammonium salt (Amersham, Arlington Heights, Ill.) to a specific activity of at least 103 to 106 cpm/µg, and Southern hybridization⁸ was performed. The membrane was autoradiographed for 4 hours at -70° C with Kodak X-OMAT AR film (Eastman Kodak, Rochester, N.Y.). The autoradiograph was developed in a Kodak X-OMAT M20 automated processor (Eastman Kodak).

Results

In Fig. 2, lane 1, labeled varicella zoster virus shows a 169 bp band, indicating that the chosen primers amplified the desired product. Two bands of higher molecular weight in this lane represent nonspecific hybridization of viral DNA. Lane 2 contains an unrelated sample. The lane labeled WHF contains the products of reactions with concurrently processed tissue culture cells. The lack of discernible band indicates that the primers chosen for amplification of viral DNA do not amplify equivalent amounts of human genomic DNA. Importantly, the absence of a band also indicates that the DNA samples were not contaminated with exogenous viral DNA during the extraction process and subsequent handling. The lanes labeled CVS 1 and CVS 2 both contain the desired 169 bp fragment of DNA, suggesting that varicella zoster virus DNA was present in both chorionic villus sampling samples.

Comment

Fetal varicella syndrome is characterized by cicatrix formation, growth retardation, limb defects, cortical atrophy, chorioretinitis, and microcephaly. This syndrome is distinct from that of neonatal varicella acquired from a third-trimester maternal varicella infection. In fetal varicella syndrome defects have been attributed to maternal varicella zoster virus infection occurring in the first and second trimesters. Laforet and Lynch⁹ first reported a case of fetal varicella syndrome in 1947. Retrospective studies have suggested a 15% to 20% incidence of fetal varicella syndrome after a primary maternal infection. Prospective studies have suggested an attack rate of 4%.^{1, 10}

Prenatal detection of varicella infection has been described. In one report, cordocentesis was used in a 32-week hydrocephalic fetus to detect varicella zoster virus—specific immune globulins.⁴ A second recently presented case described ultrasonographic findings at 30 weeks' gestation compatible with fetal varicella syndrome with intrauterine growth retardation and hydramnios, developing after maternal varicella at 15 weeks.³

In utero infections may arise as a result of hematogenous spread, ascending infection, or even direct extension. ^{10, 11} Decidua, amniotic fluid, secundines, and the fetus may all be infected. It is possible for some, all, or none of these compartments to be infected at any given time in the course of illness. The actual sequence of events has clinical relevance as to the timing, frequency, and route of clinical diagnostic procedures. Local defense factors arising from decidua and amniotic fluid, such as cytokines and immune globulins, may act to suppress infection and thus modify the course of illness. The fetus may generate immune globulins at 20 to 24 weeks' gestation. Of note, certain fetuses with

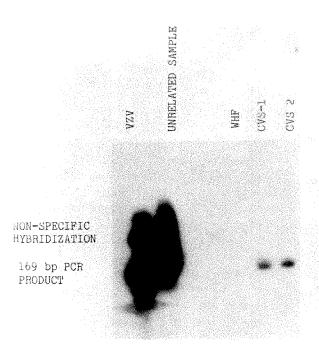


Fig. 2. Southern blot. *Lane 1*, labeled *VZV* (varicella zoster virus), shows 169 bp positive control band. Two bands of higher molecular weight in this lane represent nonspecific hybridization of viral DNA. *Lane 2* contains unrelated sample. Lane *WHF* (whole human fibroblasts) constitutes a negative control. Lanes *CVS 1* and *CVS 2* both contain desired 169 bp fragment of DNA, suggesting that varicella zoster virus DNA was present in both chorionic villus sampling samples.

congenital infections such as rubella have not developed an immunoglobulin M response until 22 to 24 weeks' gestation. The implications, then, for fetal umbilical blood sampling by cordocentesis are that reliable test results may not be available until after 24 weeks, an issue of critical importance for those patients considering termination of pregnancy.

The pathophysiologic characteristics of fetal infections and the variability of clinical expression require further elucidation.^{12, 13} In the case of varicella zoster virus infection the incidence of clinically evident structural defects after fetal infection is not known. Virus may remain latent in ganglia¹⁴ and damage may arise from recurrence.¹⁵ Also unknown are the long-term consequences of infections that do not cause in utero malformations. The analogous situation is the case of in utero cytomegalovirus infection, where neurologic sequelae such as sensorineural hearing loss may develop in structurally normal, but infected, infants.

The current preliminary findings point to molecular tools such as polymerase chain reaction¹⁶ that may prove helpful in extending our understanding of the consequences to the fetus of maternal varicella during pregnancy. Taking care to use concurrent control tissues and to prove the identity of polymerase chain reaction amplification products by Southern blot hybrid-

ization, we proved the presence of varicella zoster virus DNA in two chorionic villus sampling specimens with primer pairs specific to the varicella zoster virus inverted repeat short element containing open reading frame 63 (Fig. 2). The existence of these viral sequences in the tissues documents placental infection but cannot reveal the extent or manifestations of that infection. Furthermore, the mere presence of detectable varicella zoster virus DNA does not indicate continuing viral replication, because truly latent virus is similarly detectable, although the only currently recognized site of varicella zoster virus latency is sensory nerve ganglia and not tissues harvested by chorionic villus sampling. At present, polymerase chain reaction is a promising research tool to investigate the pathophysiologic characteristics of perinatal infections.

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Prenatal diagnosis with fetal cells isolated from maternal blood by multiparameter flow cytometry

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A long-sought goal of medical genetics has been development of prenatal diagnostic procedures that do not endanger the conceptus. Reliable and universal screening for cytogenetic disorders would require analysis of fetal cells isolated from the maternal circulation. This would be applicable to all pregnant women, irrespective of their ages or histories. In the current study fetal nucleated erythrocytes were flow sorted on the basis of four parameters: cell size, cell granularity, transferrin receptor, and glycophorin-A cell surface molecule. By polymerase chain reaction with oligonucleotide primers flanking single-copy Y-specific deoxyribonucleic acid sequences, male fetuses were correctly identified among flow-sorted samples in 12 of 12 (100%) pregnancies; female fetuses were correctly identified in 5 of 6 (83%) pregnancies. We also achieved the prenatal diagnosis of fetal aneuploidies by use of flow-sorted nucleated fetal erythrocytes and in situ hybridization with chromosome-specific deoxyribonucleic acid probes: one case of trisomy 21 that was detected in maternal blood taken 1 week after chorionic villus sampling and one case of trisomy 18 that was detected in maternal blood taken immediately before chorionic villus sampling. Although our results are promising, additional data on the background sensitivity and specificity of in situ hybridization in flow-sorted fetal cells will be necessary to minimize subjective interpretation and permit clinical application. (Am J Obstet Gynecol. 1991;165:1731-37.)

Key words: Fetal cells, prenatal diagnosis

A long-sought goal of medical genetics has been development of prenatal diagnostic procedures that do not endanger the conceptus. Amniocentesis and chorionic villus sampling are currently used for recovery of fetal cells for prenatal diagnosis, but these are invasive techniques carrying small, yet finite, risks to the developing fetus. The possibility of noninvasive methods for prenatal diagnosis is especially attractive. This would be applicable to all pregnant women, irrespective of their ages or histories. Indeed, an element of noninvasive screening already exists; concomitant use of maternal age, maternal serum α -fetoprotein, unconjugated estriol level, and human chorionic gonadotropin identifies 60% of pregnancies involving fetal Down

syndrome.¹ Couples determined to be at increased risk are then offered invasive procedures such as amniocentesis. Attempting to delineate ultrasonographic criteria for detecting fetal Down syndrome is another example.²

Reliable and universal screening for cytogenetic disorders would require analysis of fetal cells isolated from the maternal circulation. Indeed, obstetricians and pathologists have long known that occasional fetal cells find their way into the maternal circulation, as indicated by the phenomena of rhesus immunization and amniotic fluid embolization. In 1969 Walknowska et al.³ first proposed recovering fetal cells from maternal blood for prenatal diagnosis.

We sought to determine whether fetal nucleated erythrocytes could be sorted from maternal blood during the first and second trimesters by multiparameter flow cytometry and confirmed this with polymerase chain reaction amplification of oligonucleotide primers flanking single-copy Y-specific deoxyribonucleic acid (DNA) sequences. We also report here the first-trimester prenatal diagnoses of fetal aneuploidy with the use of fetal nucleated erythrocytes that have been flow sorted from maternal blood and analyzed by in situ hybridization with chromosome-specific DNA probes: one case of trisomy 21 that was diagnosed from maternal blood taken 1 week after chorionic villus sam-

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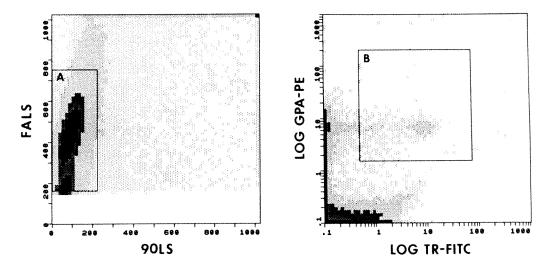


Fig. 1. Flow cytometric scattergrams showing gates for cell sorting. **A,** Forward-angle light scatter (*FALS*) for cell size; 90-degree light scatter (*90LS*) for cell granularity. **B,** Glycophorin-A monoclonal antibody conjugated to phycoerythrin (*GPA-PE*); transferrin receptor monoclonal antibody conjugated to fluorescein isothiocyanate (*TR-FITC*).

pling and one case of trisomy 18 that was diagnosed from maternal blood taken immediately before chorionic villus sampling.

Methods

Specimens and sample preparation. Specimens were taken from volunteers giving informed consent under a University of Tennessee, Memphis, Institutional Review Board—approved protocol. Immediately before chorionic villus sampling or amniocentesis approximately 20 ml venous blood was aspirated into vacuum tubes containing acid-citrate-dextrose solution A. Each blood sample was logged for reference to cytogenetic analysis and coded. Blood samples stayed in the solution for up to 36 hours before preparation and sorting. In one case blood was drawn 1 week after chorionic villus sampling diagnosis of fetal trisomy 21. Umbilical cord blood from term pregnancies served as controls.

Four milliliters of blood diluted 1:1 with phosphate-buffered saline solution was layered over 3 ml of chilled Ficoll-Paque (Pharmacia, Piscataway, N.J.) and centrifuged at 350g for 40 minutes at room temperature. All buoyant mononuclear cells, early reticulocytes, and small numbers of erythrocytes were removed by suction and washed twice with phosphate-buffered saline solution. The yield ranged from 2 to 3×10^7 cells per sample.

After washing, the cells were resuspended in phosphate-buffered saline solution with 2% fetal bovine serum and 0.1% sodium azide (buffer). The cells were counted and the volume adjusted to a concentration of 5×10^6 cells per milliliter. Cells were dual labeled for 30 minutes at 4° C with transferrin receptor monoclonal antibody conjugated to fluorescein isothiocyanate (T9-

FITC, Coulter Immunology, Hialeah, Fla.) and glycophorin-A monoclonal antibody conjugated to phycoerythrin (KC16-Rd¹, Coulter Immunology). The final concentration was $0.2~\mu g$ per 1 million cells for each antibody. Labeled cells were washed once, resuspended at 10^7 cells per milliliter in buffer, and analyzed and sorted by flow cytometry (as described).

Flow cytometry. Cells were sorted with an EPICS Elite flow cytometer (Coulter Cytometry, Hialeah) with standard instrument computer and electronics. The unit was configured with an air-cooled 15 mW argon laser (model 2201, Cyonics, San Jose, Calif.) emitting at 488 nm. Elite 2.1 cytometry software was used. Cells were analyzed in a single pass at a rate of 2500 to 5000 cells per second. Positive selection was based on forward scatter (cell size), side scatter (cell granularity), green fluorescence (transferrin receptor, T9-FITC), and orange fluorescence (glycophorin-A, KC16-Rd¹). Sort gates for positive cell selection are shown in Fig. 1. Dual fluorescent cells of appropriate size and granularity were sorted directly into a 1.5 ml Eppendorf tube containing 1 ml of buffer.

Polymerase chain reaction. For polymerase chain reaction sorted fetal cells were centrifuged at 10,000g for 3 minutes and the buffer removed. Tubes containing the cells were stored frozen at -85° C pending batch analysis. We used the dual-amplification ("nested primer") polymerase chain reaction method described by Lo et al.⁴ with oligonucleotide primers flanking a single-copy Y-chromosome-specific DNA sequence. The final product was run in 4% agarose gel and stained with ethidium bromide for visualization and scoring. For details of the procedure, see Wachtel et al.⁵

In situ hybridization. The concentration of sorted

fetal cells was adjusted to 4000 to 12,000 nuceli per milliliter of phosphate-buffered saline solution containing 5% fetal bovine serum and 0.1% azide. For each slide 1000 to 3000 nuclei were plated onto poly-L-lysine-coated slides by cytocentrifugation at 550 rpm for 8 minutes (Cytospin, Shanndon, Pittsburgh). Slides were air dried, fixed by immersion in acid ethanol (containing 5% glacial acetic) for 10 minutes at room temperature, and stored dry with a desiccant at 4° C for batch processing. Immediately before hybridization, the cells were rehydrated by two washes in phosphatebuffered saline solution for 2 minutes at room temperature. All remaining washes and incubations were accomplished at room temperature unless otherwise noted.

Cells on the slides were permeabilized in phosphatebuffered saline solution with 0.05% Triton X-100 for 3 minutes, washed twice for 2 minutes with phosphatebuffered saline solution, treated with 0.1 N hydrochloric acid for 10 minutes, and again washed twice for 2 minutes with phosphate-buffered saline solution. Each slide was treated with 25 µl of 50 ng/ml proteinase K in 20 mmol/L Tris hydrochloride, pH 7.0, coverslipped, and deproteinized for 7 minutes at 37° C. Digestion was stopped by two 2-minute washes in phosphate-buffered saline solution containing 2 mg/ml glycine. Samples were fixed for 5 minutes with 4% paraformaldehyde, washed twice for 2 minutes in phosphate-buffered saline solution, and blocked for 10 minutes with 0.25% acetic anhydride in 0.1 mmol/L triethanolamine hydrochloride containing 0.9% sodium chloride, pH 8.0. The cells were dehydrated by treatment with a series of 70%, 80%, 90%, and 100% ethanol solutions (1 minute per solution), and air dried for hybridization.

In situ hybridizations were performed with chromosome-specific probes (Integrated Genetics, Inc., Framingham, Mass.): HL 10-10-8a, a repetitive sequence specific for the X centromere; pWe7.1 and pWe5.0, overlapping mid-long-arm regions specific for chromosome 18; and 519, a 20 kb sequence on the distal long arm of chromosome 21; pDP97, a 40 kb repeat sequence from the distal long arm of the Y chromosome, was provided by David Page, Cambridge, Mass. For each hybridization, probe was combined with 50 ng biotinylated cosmid DNA, 1 µg competitor human DNA (except for the Y probe), and 8.95 µg salmon sperm DNA (9.95 µg with the Y probe). Probe, competitor, and carrier DNA were precipitated with 100% ethanol, pelleted, washed with 70% ethanol, and dried in a Speed-Vac (Savant, Hicksville, N.Y.) for 5 to 15 minutes.

The sample was resuspended at a final concentration of 10 µg total DNA per 10 µl of hybridization solution consisting of 50% formamide and 10% dextran sulfate in 6× saline-sodium citrate buffer (SSC). Ten microliters of probe was placed on the sample spot; this was overlaid with a glass coverslip and sealed with rubber cement. Sample and probe DNA was denatured on a slide warmer for 7 minutes at 80° C and hybridized overnight in a moist chamber at 37° C. Each slide was washed three times for 5 minutes with 50% formamide in $0.1 \times$ SSC at 42° C, once for 5 minutes in $2 \times$ SSC at room temperature, and three times for 5 minutes in 2× SSC at 60° C. The samples were blocked with 50 μ l of 4 × SSC with 3% bovine serum albumin at 37° C for 30 minutes in a moist chamber. Cells were stained with 50 µl of 5 µg/ml avidin-fluorescein isothiocyanate in $4 \times$ SSC with 1% bovine serum albumin-0.1% Tween 20. The slides were incubated in the dark for 15 minutes at 37° C in a moist chamber, washed once for 5 minutes in 2× SSC at room temperature, and drained. Next, the slides were mounted with 20 μl of antifade-4',6-diamidino-2-phenylindole, coverslipped, and sealed with nail polish. Slides were stored at 4° C in the dark for scoring by fluorescence microscopy.

Results

Flow cytometry. Among the control umbilical cord samples sorted (n = 17), cells positive for each of the four parameters accounted for a value of $21.0\% \pm 7.6\%$ (mean \pm SD) of the mononuclear cell fraction separated over Ficoll. By contrast, cells positive for the four parameters represented only $0.4\% \pm 0.7\%$ (mean ± SD) of cells separated from first-trimester maternal samples (n = 50) and 2.2% \pm 3.6% (mean \pm SD) of cells sorted from second-trimester maternal samples (n = 20). Although many of the sorted cells were anucleate reticulocytes, we could generally retrieve 2000 to 20,000 nuclei, of which an estimated 10% were nucleated fetal erythrocytes. Thus we predicted that fetal cells should be present in frequencies of about one nucleated erythrocyte per 107to 108 maternal cells in maternal whole blood.

Polymerase chain reaction. We tested 18 sorted patient blood samples by polymerase chain reaction. Male fetuses were correctly identified in 12 of 12 (100%) pregnancies, whereas female fetuses were correctly identified in 5 of 6 (83%) pregnancies. The overall efficiency of fetal sex identification with polymerase chain reaction was thus 17 of 18 (94%) among sorted samples.

In situ hybridization. The background signal level of Y-specific probe (pDP97) was 5% in sorted female cord blood samples (Fig. 2). With 5% used as a baseline above which male sex would be predicted, fetal sex was correctly identified in 68% of samples sorted from maternal peripheral blood (6/8 females and 9/14 males).

Cases involving fetal aneuploidy. In the first case a 37-year-old patient underwent transcervical chorionic

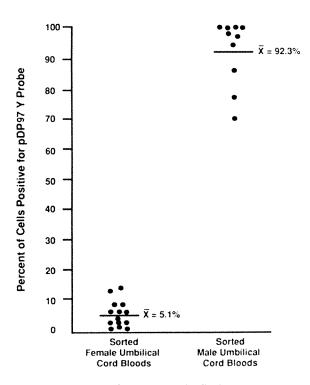


Fig. 2. Distributions of percentage of cells showing positive in situ hybridization signal with pDP97 Y-chromosome-specific DNA probe in sorted umbilical cord blood samples from female (n = 14) and male (n = 10) infants.

villus sampling at 10 weeks' gestation. Her one previous pregnancy resulted in the delivery of a normal male infant. Cytogenetic analysis revealed a 47,XX,+21 karyotype (direct cytotrophoblasts). One week after chorionic villus sampling, a maternal blood specimen was collected for flow cytometry. After being enriched for fetal cells, the sample was analyzed by in situ hybridization with probe (519), which as previously noted is specific for chromosome 21. Slides were scored by three independent observers who were unaware of the fetal karyotype. Sixty-two nuclei were examined: 13 (21%) displayed the two hybridization signals expected of normal diploid cells, but 24 of 62 (39%) displayed three signals indicating trisomy for chromosome 21 (Table I). The diagnosis of 47,XX,+21 was confirmed in abortus tissue.

In the second case a 42-year-old woman was referred for prenatal diagnosis at 10.8 weeks' gestation because of maternal age. Her three previous pregnancies resulted in the delivery of a normal female infant and the delivery of a set of normal twins, one male and one female. From a sample received before chorionic villus sampling, flow-sorted fetal cells were analyzed by in situ hybridization with probes of chromosomes, Y, X, 18, and 21 (Fig. 3). Again, samples were scored without knowledge of fetal karyotype. Results are shown in Table II: Y probe, 8.7% of the nuclei exhibited a single

hybridization signal and 90.3% exhibited no signal; X probe, 14.5% of the nuclei displayed a single hybridization signal and 86% two signals; chromosome 18 probe, 8.5% of the nuclei displayed three hybridization signals; chromosome 21 probe, 95.2% of the nuclei showed two hybridization signals. In aggregate, these data indicate a male fetus with trisomy 18. The patient elected to terminate the pregnancy on the basis of the chorionic villus sampling results, and the diagnosis of 47,XY, +18 was confirmed in the abortus tissue.

Comment

In the current study fetal nucleated erythrocytes were flow sorted on the basis of four parameters: cell size, cell granularity, transferrin receptor, and glycophorin-A. By polymerase chain reaction with oligonucleotide primers flanking single-copy Y-specific DNA sequences, male fetuses were correctly identified among flow-sorted samples in 12 of 12 (100%) pregnancies and female fetuses were correctly identified in 5 of 6 (83%) pregnancies. The estimated ratio of fetal nucleated erythrocytes to maternal cells was 1:107 to 1:108. Multiparameter flow cytometry enabled sorting enrichment of approximately one fetal nucleated erythrocyte per 10 to 20 nucleated maternal cells. With flowsorted cells and in situ hybridization with a Y-chromosome-specific DNA probe, fetal sex was correctly predicted in 15 of 22 (68%) pregnancies.

Our findings help confirm the possibility of recovering fetal cells from maternal blood for prenatal diagnosis, a thesis first raised in 1969 by Walknowska et al.3 These workers described XY metaphases in maternal blood of pregnant women carrying a male fetus. Although subsequent reports suggested that fetal cells could be detected and isolated from maternal blood, 6-8 skepticism remained. More recently, however, several groups have made significant advances. Lo et al.4.9 studied unsorted blood from pregnant women. Polymerase chain reaction was performed, with nested primers used for a Y sequence. Women carrying a male fetus proved far more likely to show a hybridization signal than those carrying a female fetus. Concurrently, Mueller et al.,10 using a different approach, screened 6000 monoclonal antibodies generated from placental tissue; five were found to be specific for fetal tissue. After these monoclonal antibodies were exposed to maternal blood from pregnant women, isolated cells were subjected to polymerase chain reaction for Y sequences. Fetal sex was correctly identified in seven of seven males and six of seven females. Bianchi et al.11 flow sorted on the basis of transferrin-receptor-positive cells and used polymerase chain reaction to amplify for Y sequences. Of eight samples showing the Y sequence, six were from pregnancies in which women were carrying male fetuses. Finally, Yeoh et al.12 sorted fetal cells for a pa-

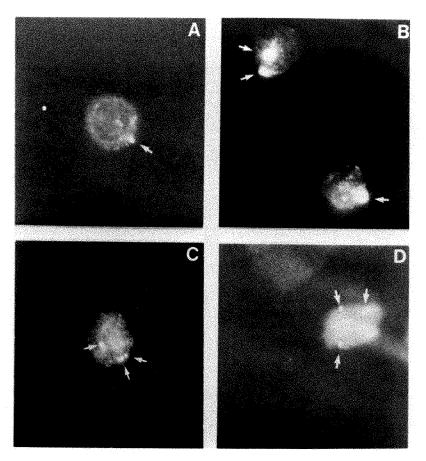


Fig. 3. Flow-sorted cells hybridized to various chromosome-specific probes. Probes were labeled with biotin and visualized with fluorescein isothiocyanate-streptavidin. All cells were from a single blood sample and analyzed in coded fashion. A, Male cell of fetal origin. Fluorescent signal (arrow) in cell hybridized to pDP97 probe, which identifies repetitive sequence in heterochromatic region on long arm of Y chromosome. B, Female cell of maternal origin (upper left) showing two signals (two arrows) and male cell of fetal origin (lower right) showing single signal (arrow). Cells hybridized to X-chromosome-specific cosmid probe, which identifies pericentromeric repeat sequence on X chromosome. C and D, Trisomy 18 fetal cells sorted from maternal blood. Fluorescent signals (three arrows) in cells hybridized to chromosome 18-specific cosmid contig probe, which identifies single-copy sequence on distal long arm of chromosome 18.

Table I. Flow-sorted maternal blood specimen obtained after transcervical chorionic villus sampling in woman carrying 47,XX, +21 fetus: Distribution of in situ hybridization signals for chromosome 21specific probe (519)

			No. of hybridization si	gnals per nucleus		
	0	1	2	3	4	Total
No. of nuclei	3 (4.8%)	14 (22.6%)	13 (21.0%)	24 (38.7%)	8 (12.9%)	62

ternal HLA allele not present in the mother, subjected sorted cells to polymerase chain reaction for a Y sequence, and again found encouraging results with respect to predicting fetal sex. These studies suggest that fetal cells indeed exist in maternal blood. However, polymerase chain reaction will rarely be diagnostic; instead, other technologies will be necessary for this

To this end, this is the first demonstration of prenatal

detection of fetal aneuploidies by use of flow-sorted nucleated fetal erythrocytes and chromosome-specific DNA probes. In one case in which a blood sample was obtained 1 week after transcervical chorionic villus sampling, 24 of 62 (38.7%) cells showed three hybridization signals with a chromosome 21-specific DNA probe; this indicated fetal trisomy 21. We have previously shown that fetal cells are usually transferred to maternal circulation as result of chorionic villus sampling,13 and we

Table II. Flow-sorted maternal blood specimen obtained before transabdominal chorionic villus sampling in woman carrying 47,XY, +18 fetus: Distribution of in situ hybridization signals for chromosome-specific probes—Y chromosome (pDP97), X chromosome (HL10-10-8a), chromosome 18 (pWe7.1 and pWe5.0), and chromosome 21 (519)

		No. of hyb	ridization signals per nucle	us	
Probe	0	1	2	3	Total
Y	93 (90.3%)	9 (8.7%)	1 (1.0%)	- Carpinature	103
X		17 (14.5%)	100 (85.5%)		117
18	resolver	16 (5.4%)	254 (86.1%)	25 (8.5%)	295
21		3 (1.8%)	158 (95.2%)	5 (3.0%)	166

suspect that the large number of trisomic cells in this case represents such a transfer. That 8 of 62 nuclei (12.9%) showed four signals with the 21-specific probe could indicate either tetrasomy reflecting transfer of multinucleated trophoblast cells or, alternatively, inclusion of dividing maternal or fetal cells in the sorted population. Other potential variables are discussed in the text that follows. In the second and more noteworthy case, a blood sample was taken before chorionic villus sampling. The combination of results from X and Y probes indicated that approximately 10% of the sorted cells were from a male fetus, the remaining 90% of cells being of maternal origin. Approximately 9% of sorted cells showed three hybridization signals with the chromosome 18-specific DNA probe. In aggregate, these data indicate a male fetus with trisomy 18. In both cases the chromosome abnormalities were confirmed in the abortus specimens.

A variety of factors have been shown to influence hybridization efficiency and signal specificity.14.15 Hybridization or detection efficiency, in particular, has an impact on the enumeration of interphase chromosomes, as does the geometry or chromosomal location within the nucleus. Thus in any population of disomic cells, whereas the majority (≥90%) of cells display two hybridization signals per chromosome, a small number display one, three, or four signals. A single signal most likely results from incomplete detection of both chromosomes because of inefficient hybridization-detection or overlap in the nucleus of the two chromosomal domains. Four hybridization signals arise from G2 nuclei, with three-signal nuclei representing incomplete detection of all four copies of the chromosome.14 Similar effects on detection of chromosomes are seen in trisomic cells and probably account for the detection of four-signal nuclei in case 1 above. The microscopic resolution of contiguous signals and focal planes of a signal that is unclear in one plane may become clear on refocusing the microscope and vice versa. Other variables include (1) the cell type being analyzed, (2) the specific probe used, and (3) the conditions under which the cells are manipulated (e.g., flow sorted). It has been shown that minor alterations in protocol can significantly influence both the qualitative and quantitative aspects of intranuclear trisomy detection. ¹⁴ Protocols must be optimized for each cell type. ¹⁶ A systematic study of the variables with the use of the flow-sorted fetal cells is under way in our laboratories to determine the sensitivity and specificity of in situ hybridization for identification of chromosomal abnormalities in flow-sorted fetal cells.

In summary, our preliminary in situ hybridization data are encouraging, demonstrating the real possibility for prenatal diagnosis of cytogenetic abnormalities with fetal cells isolated from maternal blood. Although promising, additional data on the background sensitivity and specificity of in situ hybridization in flow-sorted fetal cells will be necessary to minimize subjective interpretation and permit clinical application. If these issues are successfully addressed, we would envision this method being used initially as a screening test with confirmation of abnormal results by conventional invasive techniques such as chorionic villus sampling or amniocentesis. Eventually, fetal cells isolated from maternal blood would be used for definitive fetal diagnosis. Methods such as these would enable a couple to undergo prenatal diagnosis without endangering the fetus and open prenatal diagnosis to all couples irrespective of family history or traditional risk factors such as advanced maternal age.

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Absence of hyperinsulinemia in isoimmunized fetuses treated with intravascular transfusion

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We serially sampled blood from fetuses of five severely isoimmunized pregnancies at the time of each intrauterine intravascular transfusion and at birth. We were unable to demonstrate either an elevation in the plasma insulin/glucose ratio or a relationship between the insulin/glucose ratio and hemoglobin concentration at any time period. Plasma total glutathione concentration, however, decreased dramatically from the initial to the second transfusion (323 \pm 114 to 43 \pm 9 ng/ml; t=-5.06, p<0.01). We speculate that intrauterine transfusion may modify or prevent the previously reported fetal pancreatic β -cell hyperplasia and hyperinsulinemia associated with isoimmunization by decreasing red blood cell hemolysis and thereby circulating glutathione. (AM J OBSTET GYNECOL 1991;165:1737-40.)

Key words: Fetal hyperinsulinemia, isoimmunization, intrauterine transfusion

Isoimmunized fetuses have been reported to be at risk for neonatal hypoglycemia presumably as a secondary effect of hyperinsulinemia developing in utero.^{1, 2} Additionally, hyperreactivity of insulin secre-

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tion has been noted in severely affected neonates after exchange transfusion with citrated blood or after an intravenous glucose injection. These clinical observations are supported by the pathologic identification of hyperplasia of the islets of Langerhans in infants who die of erythroblastosis fetalis. 4-6

It has been proposed that the degree of fetal hyperinsulinemia is related to the severity of fetal anemia.^{8,7} One hypothesized mechanism for the fetal hyperinsulinism is an impairment of the effect of circulating insulin by glutathione liberated from hemolysis of red blood cells.^{8,9} Destruction or inactivation of circulating insulin might then lead to compensatory hy-

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Table I. Measurements at each transfusion and birth

Sensitizing antigen	Gestational age (wk)	Hemoglobin (gm/dl)	Glucose (mg/dl)	Insulin (μU/ml)	Insulin†glucose ratio	Glutathione (ng/nl)
D	22	8.2	71	2.3	0.03	513
	24	7.8	69	3.5	0.05	43
	27	8.8	128	22.8	0.18	_
	31	8.4	98	19.6	0.20	_
	35	10.9	51	3.5	0.07	
D	25	7.1	97	3.3	0.03	385
	27	12.9	128	39.3	0.31	57
	30	8.0	114	45.0	0.39	_
	34	8.2	130	32.5	0.25	_
	36	8.0	85	8.2	0.10	
D	27	4.8	104	3.3	0.03	287
	29	8.9	145	12.2	0.08	46
	33	9.1	100	7.8	0.08	
D	28	4.1	69	7.0	0.10	194
	31	7.3	69	9.5	0.14	31
	36	7.8	81	4. l	0.05	
c, Kell	27	5.7	76	5.0	0.07	237
-,	29	9.3	70	3.6	0.05	40
	32	*****				_
	35	10.8	84	8.8	0.11	_

perplasia of the islets of Langerhans. To investigate the relationship between fetal anemia and hyperinsulinemia, we serially measured fetal insulin, glucose, glutathione, and hemoglobin in fetuses of five severely isoimmunized pregnancies at the time of intrauterine intravascular transfusion and at birth.

Material and methods

Our technique for ultrasonographically guided intrauterine straight transfusion has been described previously. 10 Briefly, the placental insertion of the umbilical cord is identified by sector real-time ultrasonography, after which the abdomen is aseptically prepared before local infiltration with lidocaine. A complete surgical scrub is not performed although the operators wear a cap, mask, and gloves. A 22-gauge, 5-inch disposable spinal needle is then guided toward the umbilical cord (preferably the umbilical vein at the placental insertion site) with a 5 MHz sector transducer. After blood is aspirated for the necessary studies, pancuronium bromide (0.05 to 0.1 mg/kg) is infused to paralyze the fetus. Throughout the transfusion the fetal heart rate is intermittently visualized and patients ≥26 weeks' gestation have an intravenous infusion to respond to a possible nonremediable fetal bradycardia. Neither prophylactic antibiotics nor tocolytic drugs are adminis-

This study was approved by the Northwestern University Institutional Review Board and required <1 ml of additional fetal blood to be aspirated before the beginning of each intrauterine transfusion or from umbilical venous cord blood at birth. A double-antibody radioimmunoassay was used to measure plasma insulin with human insulin as the standard. Plasma total glu-

tathione concentrations were measured with a modified enzymatic microassay.¹² The interassay coefficients of variation were 9% for insulin and 10% for glutathione. Plasma glucose concentrations were determined with an automated analyzer (Beckman II) and hemoglobin was measured in a colorimeter with a cyanmethemoglobin reaction (model ELT-800 hematology analyzer or Coulter Counter).

Linear regression by means of a least-squares method was performed to analyze the relationship between fetal hemoglobin and either plasma insulin or the insulin/glucose ratio. Analysis of variance was used to investigate any trend in the insulin/glucose ratio from the initial transfusion until birth. Comparison of glutathione levels at the first and second intrauterine transfusions was performed by paired t test. Statistical significance for all measures was assumed at the p < 0.05 level.

Results

The sensitizing antigen, timing of each transfusion and birth, and measurements of hemoglobin, glucose, insulin, insulin/glucose ratio, and glutathione for each of the five severely isoimmunized fetuses are listed in Table I. Patient 5 had one additional intrauterine transfusion at 32 weeks' gestation, but insufficient fetal blood was aspirated for research studies. All five patients had a negative screen for gestational diabetes mellitus between 24 and 28 weeks' gestation (plasma blood glucose level <130 mg/dl 1 hour after an oral 50 gm glucose load). Four patients were delivered by cesarean section; three were elective repeat operations but patient 3 was delivered by emergency cesarean section because of a fetal bradycardia during her third intrauterine trans-

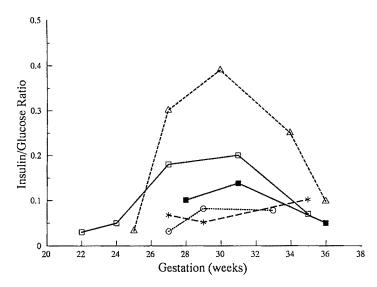


Fig. 1. Insulin/glucose ratios are depicted for each fetus at each intrauterine transfusion. Whether data are analyzed according to first transfusion, second transfusion, and birth or at 26 to 28 weeks, 29 to 31 weeks, and birth, there were no significant differences over time (analysis of variance, p > 0.05).

Table II. Serial measurements (mean \pm SD) obtained for all fetuses (n = 5)

	Glucose	Insulin	Insulin/glucose	Hemoglobin
	(mg/dl)	(µU/ml)	ratio	(gm/dl)
First transfusion	83.4 ± 14.3	4.2 ± 1.7	0.05 ± 0.03	6.0 ± 1.5
Second transfusion	96.2 ± 33.3	13.6 ± 13.2	0.13 ± 0.10	9.2 ± 2.0
Birth	80.2 ± 16.0	6.5 ± 2.2	0.08 ± 0.02	9.3 ± 1.3

fusion. The pretransfusion fetal blood sample in this case was taken before fetal compromise and was used for the birth measurements. The range of birth weight was 2020 to 2925 gm. Four of the neonates had an Appar score of >7 at 5 minutes; that neonate who was delivered by emergency cesarean section had a score

The measurements (mean ± SD) obtained before the initial transfusion, before the second transfusion, and at birth are displayed in Table II. Although fetal transfusions were performed at differing gestational ages for the five pregnancies studied, each fetus was transfused at 26 to 28 weeks and again at 29 to 31 weeks. Mean values at 26 to 28 weeks for plasma insulin and the insulin/glucose ratio were 15.5 \pm 13.8 μ U/ml and 0.14 ± 0.10 ; at 29 to 31 weeks these were 18.0 ± 14.5 μ U/ml and 0.17 \pm 0.12, respectively.

There were no identifiable relationships between fetal hemoglobin and either plasma insulin or the insulin/glucose ratio at any time period. The insulin/glucose ratios are depicted for each fetus at each transfusion and at birth in Fig. 1 (analysis of variance, p > 0.05 for first transfusion, second transfusion, and birth, or at 26 to 28 weeks, 29 to 31 weeks, and birth).

Plasma total glutathione concentrations were mea-

sured only at the initial and second intrauterine transfusions because after this time the fetal blood volume has essentially been replaced by adult red blood cells. There was a significant decline (mean \pm 1 SD) from 323 ± 114 to 43 ± 9 ng/ml (t = -5.06, p < 0.01).

Comment

Reports in the late 1960s alerted pediatricians to the propensity for hypoglycemia to develop in neonates of isoimmunized pregnancies.1,2 Analogies were drawn to the infant of the diabetic mother because hyperplasia of the islets of Langerhans and increased extractable pancreatic insulin were documented in newborns dying of erythroblastosis fetalis.4-6,8 Additionally, elevated plasma insulin levels were observed shortly after birth3.7,13 and were noted by some authors to be inversely correlated with hemoglobin concentration, particularly in the most severely affected pregnancies.3.7

In our five severely isoimmunized pregnancies requiring intrauterine intravascular transfusions neither fetal plasma insulin levels nor the insulin/glucose ratio was related to hemoglobin concentration at any time period. At first glance fetal hyperinsulinemia was suggested because the mean plasma insulin values at 26 to 28 weeks and 29 to 31 weeks, but not at birth, approached¹⁴ or exceeded¹⁵ the upper limit of previously published 95% confidence intervals derived in utero from a population of appropriate-for-gestationalage fetuses. Our fetal insulin measurements, however, may reflect higher fetal glucose concentrations that are secondary effects of the precautionary maternal intravenous infusion that was started in case of fetal complications from the intrauterine transfusion. In contrast to insulin, mean insulin/glucose ratios at 26 to 28 weeks, 29 to 31 weeks, and birth were not elevated and the individual insulin/glucose ratios are distributed around the mean values reported at comparable gestational ages.¹⁴

It is possible that our limited number of patients precluded the identification of fetal hyperinsulinemia. The clinical observation in diabetic pregnancies of accelerated fetal somatic growth in the late second trimester suggests that the fetal pancreas is capable of increased insulin secretion by this time period.16 Alternatively, fetal hyperinsulinemia may not occur as commonly in isoimmunized pregnancies as previously reported. A third possibility, however, is that in utero treatment relatively early in gestation may modify or prevent pancreatic β-cell hyperplasia and hyperinsulinemia. Consistent with this premise is the dramatic decrease in fetal glutathione concentration observed by the time of the second intrauterine transfusion. Furthermore, there was a suggestion of a rise in insulin/glucose ratio by the second transfusion with a subsequent decline by birth, possibly depicting the interruption of a pathophysiologic sequence. Replacement of fetal blood with compatible adult cells decreases hemolysis and thereby circulating glutathione. If islet cell hyperplasia is a secondary result of peripheral destruction or inactivation of insulin by glutathione, this process may be ameliorated by intrauterine transfusion.

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Does the brachial artery doppler flow velocity waveform reflect changes in downstream impedance?

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Brachial artery Doppler flow velocity waveforms were recorded from 10 nonpregnant women and 19 women with uncomplicated pregnancies. The pregnant group showed higher peak systolic frequencies (3.24 compared with 2.26, p=0.01), higher maximum end-diastolic frequencies (0.62 compared with 0.25, p=0.01), and lower pulsatility indices (2.57 compared with 3.95, p=0.06) when compared with the nonpregnant group. These Doppler changes could reflect the increase in maternal cardiac output and decline in systemic vascular resistance that occur in normal pregnancy. The effect on brachial artery Doppler of acutely increasing downstream impedance by hand immersion in ice-cold water was studied in nine women with uncomplicated pregnancies at 32 to 38 weeks' gestation. Immersion for 15 seconds was associated with a significant reduction in peak systolic frequencies (from 3.06 to 1.97, $p \le 0.005$) and maximum end-diastolic frequencies (from 0.82 to 0.03, $p \le 0.005$) and a significant increase in the pulsatility index (from 1.87 to 5.62, $p \le 0.005$). These changes persisted for the duration of immersion (60 seconds). After immersion, the brachial artery flow velocity waveform returned to its preimmersion pattern by 60 seconds. We conclude that in normal pregnancy, the maternal brachial artery Doppler flow velocity waveform reflects acute and chronic changes in downstream impedance. (AM J OBSTET GYNECOL 1991;165:1741-4.)

Key words: Brachial artery Doppler, downstream impedance

The Doppler flow velocity pattern in vivo is influenced by a number of physiologic factors. It is generally accepted that cardiac output is an important determinant of peak systolic frequencies, whereas maximum end-diastolic frequencies are influenced by downstream impedance. Arteries feeding vascular beds of high resistance are associated with little or no end-diastolic frequencies, whereas the Doppler flow velocity waveform recorded from a low-resistance bed remains above the baseline throughout diastole.

Many complications of pregnancy are associated with abnormal maternal and fetal hemodynamics. Doppler ultrasonography is a noninvasive method of measuring changes in blood flow velocity. To date, obstetric Doppler studies have concentrated on the fetal and uteroplacental circulations. The possibility of studying Doppler blood flow changes in the maternal peripheral vascular system in pregnancy has received scant attention.

The purpose of this study was to investigate Doppler blood flow changes in the maternal brachial artery during normal pregnancy and the effect on the brachial artery flow velocity waveform of an acute increase in downstream impedance.

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Methods

The effect of normal pregnancy on the maternal brachial artery Doppler waveform pattern. Brachial artery Doppler flow velocity waveforms were recorded from 10 healthy, nonpregnant women and 19 pregnant women. The pregnant group consisted of nine women with uncomplicated pregnancies between 8 and 13 weeks' gestation and 10 women with uncomplicated pregnancies between 32 and 38 weeks' gestation. Doppler recordings were obtained from the right brachial artery as it traversed the brachial fossa. Women were positioned in a semirecumbent position and allowed to rest for 15 minutes before Doppler recordings were obtained. Flow velocity waveforms were recorded with a Vasoflo 3 continuous-wave Doppler scanner with a 4 MHz probe and a 100 Hz wall filter. Doppler recordings were obtained by the same operator. The angle between the Doppler probe and the skin of the brachial fossa was adjusted until arterial Doppler waveforms with a clearly defined maximum velocity profile were obtained without interference from brachial venous Doppler signals.

To study the variability of the brachial artery Doppler flow velocity waveform with time, flow velocity waveforms were recorded for a period of 10 seconds every minute for 5 consecutive minutes. At each recording period, five Doppler flow velocity waveforms were quantified by measuring peak systolic frequency, maximum end-diastolic frequency, and pulsatility index.⁴ The Doppler measurements and maternal heart rate

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Table I. Mean ± SEM for brachial artery Doppler indexes and maternal heart rate in nonpregnant women compared with normal pregnancies

		Pregnant		
	Nonpregnant (n = 10)	First trimester (n = 9)	Third trimester $(n = 10)$	
Peak systolic frequencies	2.26 ± 0.02	3.24 ± 0.27*	2.85 ± 0.21	
End-diastolic frequencies	$0.25~\pm~0.06$	0.18 ± 0.05	$0.62 \pm 0.11\dagger\ddagger$	
Pulsatility index	3.95 ± 0.62	4.64 ± 0.30	$2.57 \pm 0.30 \dagger$	
Maternal heart rate	76 ± 3	78 ± 2	86 ± 3‡§	

^{*}p = 0.01, unpaired t test, first trimester compared with nonpregnant.

were automatically calculated by the Vasoflo 3 spectrum analyzer. Mean values of peak systolic frequency, end-diastolic frequency, pulsatility index, and heart rate for the five waveforms were calculated. Average values for the 5 minutes studied were then calculated together with a coefficient of variation. Brachial artery Doppler peak systolic frequency, end-diastolic frequency, pulsatility index, and maternal heart rate for the non-pregnant group were compared with values obtained from the pregnant women.

The effect of an acute increase in downstream impedance on the maternal brachial artery Doppler flow velocity waveform in normal pregnancy. The effect of an acute increase in downstream impedance on the maternal brachial artery flow velocity waveform was studied in nine women with uncomplicated pregnancies at a gestational age between 32 and 38 weeks. Subjects were positioned in a semirecumbent position, and, after a 15-minute rest period, the right brachial artery Doppler flow velocity waveform was recorded for 5 seconds. The subject's right hand and wrist were then immersed in ice-cold water for 60 seconds. Brachial artery flow velocity waveforms were recorded for 5 seconds at intervals of 15, 30, and 60 seconds during immersion and at 30 and 60 seconds immediately after immersion. For each recording period five flow velocity waveforms were analyzed, and mean values for peak systolic frequency, end-diastolic frequency, pulsatility index, and maternal heart rate were calculated.

The use of continuous-wave Doppler ultrasonography to study brachial artery blood flow changes in preg-

Table II. Coefficients of variation for brachial artery Doppler indices and maternal heart rate

		Pregnant		
	Nonpregnant $(n = 10)$	First trimester (n = 9)	Third trimester (n = 10)	
Peak systolic frequencies	11.5	9	19*	
End-diastolic frequencies	43.5	80	67	
Pulsatility index	21	25	41*†	
Maternal heart rate	5	5	6	

^{*}p = 0.03, unpaired t test, third trimester compared with first trimester.

nancy had been approved by the University of Glasgow ethical committee, and all patients recruited to the study gave informed consent. Student t test and Wilcoxon signed rank test were used for statistical analysis.

Results

Table I shows mean values for brachial artery peak systolic frequency, end-diastolic frequency, pulsatility index, and maternal heart rate in the three patient groups (i.e., nonpregnant, first trimester of pregnancy, and third trimester of pregnancy). Women in the first trimester of pregnancy showed significantly higher peak systolic frequencies (p = 0.01), compared with those of the nonpregnant group. Brachial artery maximum end-diastolic frequencies were significantly higher in women in the third trimester of pregnancy than in either the first-trimester group (p < 0.005) or the nonpregnant group (p = 0.01). Pulsatility index was significantly lower in the third-trimester group than in the first-trimester group (p < 0.005). Pulsatility index in the nonpregnant group was similar to that of the first-trimester group but showed higher values (not statistically significant, p = 0.06) when compared with the third-trimester group. Women in the third trimester of pregnancy had a significantly higher heart rate compared with that of the other two study groups (p < 0.05).

Table II shows the coefficients of variation for brachial artery peak systolic frequency, end-diastolic frequency, pulsatility index, and maternal heart rate for the three groups compared in Table I. Maximum end-diastolic frequencies showed the highest coefficients of variation, and maternal heart rate showed the lowest coefficients of variation. Statistical differences were present in the coefficients of variation for peak systolic frequencies (first trimester compared with third trimes-

 $[\]dagger p < 0.005$, unpaired t test, third trimester compared with first trimester.

tp = 0.01, unpaired t test, third trimester compared with nonpregnant.

p < 0.05, unpaired t test, third trimester compared with first trimester.

 $[\]dagger p < 0.01$, unpaired t test, third trimester compared with nonpregnant.

Table III. Effect of cold water pressor test on brachial artery Doppler indexes and maternal heart rate in the third trimester of 10 uncomplicated pregnancies (mean ± SEM)

	Before		During immersion		After in	nmersion
	immersion	15 sec	30 sec	60 sec	30 sec	69 sec
Peak systolic frequencies End-diastolic frequencies	3.06 ± 0.36 0.82 ± 0.18	1.97 ± 0.23* 0.03 ± 0.01*	1.94 ± 0.02 0.05 ± 0.02	2.03 ± 0.17 0.08 ± 0.04	2.66 ± 0.28† 0.45 ± 0.15†	2.92 ± 0.38 0.73 ± 0.22‡
Pulsatility index * Maternal heart rate	1.87 ± 0.21 84 ± 4	$5.62 \pm 0.61*$ 87 ± 4	4.73 ± 0.38 90 ± 2	4.71 ± 0.63 89 ± 5	$3.07 \pm 0.54 \dagger 84 \pm 3 \S$	$2.23 \pm 0.34 \ddagger 83 \pm 3$

^{*} $p \le 0.005$, Wilcoxon test for paired data, 15 seconds after immersion compared with before immersion.

ter, p = 0.03) and pulsatility index (nonpregnant compared with third trimester, p < 0.01).

Table III shows the changes in brachial artery peak systolic frequency, end-diastolic frequency, pulsatility index, and maternal heart rate during cold water immersion of the hand and wrist during the third trimester of pregnancy. Cold water immersion for 15 seconds was associated with a significant fall in brachial artery peak systolic frequencies and maximum end-diastolic frequencies ($p \le 0.005$) and a significant rise in the pulsatility index (p < 0.005). These changes were maintained during the 60 seconds of immersion. After immersion, brachial artery peak systolic frequencies and maximum end-diastolic frequencies increased and the pulsatility index fell significantly ($p \le 0.01$). By 60 seconds after immersion, brachial artery Doppler parameters had returned to their preimmersion values. Maternal heart rate rose during cold water immersion and fell after immersion. Heart rate changes reached statistical significance only when readings at 60 seconds of immersion were compared with readings 30 seconds after immersion (p = 0.02).

Comment

The Doppler flow velocity waveform pattern can be defined by measuring maximum velocity signals, i.e., the envelope of the Doppler spectrum. Waveform indexes dependent on peak systolic frequencies and maximum end-diastolic frequencies have been derived as a semiquantitative method of measuring Doppler waveform changes. The pulsatility index4 is one such index. These indices are thought to be independent of the angle of insonation. Analysis of waveform indices does not provide a measure of a change in flow rate, but it can suggest a possible mechanism. In arterial Doppler flow velocity waveforms, it is thought that cardiac output is an important determinant of peak systolic frequencies, whereas end-diastolic frequencies reflect downstream impedance. The purpose of our study was to test this theory in vivo.

Normal pregnancy is associated with significant hemodynamic changes in the maternal circulation. The timing and magnitude of these changes have been studied with invasive techniques^{5,6} and more recently by the noninvasive method of Doppler ultrasonography.7.8 There is general agreement that cardiac output increases by 30% to 50% during pregnancy. Cardiac output is a function of stroke volume and heart rate. In early pregnancy the increase in cardiac output appears to be mainly due to an increase in stroke volume, whereas in later pregnancy increased cardiac output is more dependent on a rise in maternal heart rate.9 Systemic vascular resistance, which is directly proportional to mean arterial pressure and inversely proportional to cardiac output, decreases during pregnancy. It has been suggested that this fall observed in normal pregnancy is related to decreased vascular resistance in the uteroplacental and pulmonary circulation.10

In the first part of our study we investigated the effect of pregnancy on the brachial artery flow velocity waveform pattern. In the first trimester peak systolic frequencies increased significantly. This change is compatible with the increase in maternal cardiac output normally seen in early pregnancy. Brachial artery Doppler flow velocity waveforms from women in the third trimester showed significantly higher end-diastolic frequencies compared with women in the first trimester and nonpregnant women. This change would be consistent with the fall in peripheral vascular resistance observed in normal pregnancy. We recognize that peak systolic frequencies and end-diastolic frequencies are dependent on the angle of insonation of the ultrasonographic beam. In our study with continuous-wave Doppler the magnitude of this angle was unknown. In spite of this source of variation, the changes we observed were statistically significant, and we suggest that they cannot be dismissed as due to chance. Pulsatility index is a function of peak systolic and end-diastolic frequencies and is thought to be independent of the angle of insonation. Brachial artery pulsatility index

 $[\]dagger p \leq 0.01$, Wilcoxon test for paired data, 30 seconds after immersion compared with 60 seconds' immersion.

 $[\]ddagger p \le 0.01$, Wilcoxon test for paired data, 60 seconds after immersion compared with 30 seconds after immersion.

p = 0.02, Wilcoxon test for paired data, 30 seconds after immersion compared with 60 seconds' immersion.

was significantly lower in the third-trimester group than in the first-trimester and nonpregnant groups. The fall in pulsatility index may be partly explained by the increase in maternal heart rate in the third trimester (a characteristic of normal pregnancy). However, the relationship between pulsatility index and heart rate is weak¹¹ and it is more likely that the fall in pulsatility index is a consequence of the increase in end-disatolic frequencies. It would thus appear from our study that changes in the brachial artery Doppler flow velocity waveform pattern occurred during normal pregnancy and appear to follow the expected changes in maternal cardiac output and peripheral resistance.

The variation in the brachial artery flow velocity waveform pattern with time is interesting. Previous studies of brachial artery Doppler velocimetry^{12, 13} do not remark on short-term variability. In our study the same observer performed all the Doppler recordings. The coefficients of variation for brachial artery peak systolic frequency, end-diastolic frequency, and pulsatility index ranged between 20% and 80%. In spite of this variation (particularly evident in end-diastolic frequency), a baseline pattern could be established if the vessel was insonated for a period of 5 minutes and mean values calculated. Furthermore, with this technique of analysis, significant differences in baseline measurements were found between nonpregnant women and pregnant women in the first and third trimesters of pregnancy. This would suggest that, providing a baseline Doppler pattern is established over several minutes, the brachial artery Doppler flow velocity waveform has potential as a clinical tool to study maternal cardiovascular changes.

The effect of acutely increasing downstream impedance on the brachial artery flow velocity waveform was tested by immersing the hand and wrist in ice-cold water. Brachial artery end-diastolic frequencies fell significantly by 15 seconds of immersion. During immersion (a time period of 60 seconds), end-diastolic frequencies showed no change after the 15-second recording. After immersion end-diastolic frequencies increased to reach their preimmersion range by 60 seconds. This response supports an inverse relationship

between end-diastolic frequencies in the brachial Doppler flow velocity waveform and downstream impedance.

Our findings suggest that brachial artery Doppler velocimetry may be a simple, noninvasive method of studying changes in the maternal circulation, in particular changes in peripheral vascular resistance. We postulate that this method could provide information about changes in vascular resistance in pregnancies complicated by maternal hypertension.

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Effect of pregnancy on the accuracy of light-reflection rheography

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Light-reflection rheography is a noninvasive method to detect venous obstruction by indirectly measuring venous emptying time. In nonpregnant women it has >90% sensitivity when compared with venography but has not been tested during gestation. To determine if the nonthrombotic occlusion of venous outflow by the pregnant uterus might alter the performance of light-reflection rheography, we examined 17 normal patients with a vascular Accuscan (Hemodynamics, Inc., Boca Raton, Fla.) in the third trimester of pregnancy and 11 patients during the early second trimester. These results were compared with the defined normal (nonpregnant) rate of venous emptying (slope 0.54 ± 0.06). The mean (\pm SD) for the entire sample was 0.58 ± 0.23 mm/sec in the right leg and 0.52 ± 0.19 mm/sec in the left leg measured in the standard sitting position (p = 0.21). The results did not vary with gestational age. A subset of patients in late pregnancy were used to compare the effect of various positions (supine, lateral decubitus, and sitting) on test performance. Positions other than sitting provided results that were inconsistent. It appears that the large pregnant uterus does not significantly obstruct venous outflow from the lower extremities in the standard sitting position; thus the results of light-reflection rheography are not affected. Comparison of light-reflection rheography versus venography in pregnant patients with suspected venous thrombosis is being investigated. (AM J OBSTET GYNECOL 1991;165:1745-7.)

Key words: Deep venous thrombosis, light reflection rheography

Thrombosis is six times more frequent during gestation compared with the incidence in nonpregnant patients. Deep venous thrombosis of the lower extremities complicates approximately 0.018% to 0.29% of pregnancies.1,2 Thromboembolic disease is one of the leading causes of nonobstetric death and, if untreated, will lead to a pulmonary embolus as often as 24%, with a mortality rate of $\geq 15\%$. Unfortunately, the signs and symptoms of deep venous thrombosis (muscle pain, tenderness to palpation, swelling, etc.) are often noted during normal pregnancy when there is no vascular obstruction. Noninvasive tests such as impedance plethysmography and Doppler ultrasonography can be used to diagnose a deep venous thrombosis but require welltrained and experienced personnel. Venography has been considered the gold standard for diagnosis of venous thrombosis, but it is invasive, it is associated with a significant amount of morbidity, and up to 45% of patients with suspected deep venous thrombosis will have a normal venogram.4

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Light-reflection rheography is a noninvasive method using light emitting diodes and a sensor to measure light reflected from the skin surface. The intensity of the reflected light establishes a graphic pattern that indirectly quantifies parameters of venous function by measuring changes in microcirculation. Light-reflection rheography has been shown to be as accurate as other noninvasive tests in the diagnosis of deep thrombosis in nonpregnant patients.5,6 Because the gravid uterus may decrease the venous outflow from the lower extremities, the usefulness of light-reflection rheography in the pregnant patient is questionable. The purpose of this study was to determine if light-reflection rheography during gestation would perform as consistently as had been demonstrated in patients who were not pregnant.

Material and methods

In this prospective study patients in the second and third trimesters were evaluated by light-reflection rheography during a prenatal visit. Inclusion criteria were (1) intrauterine pregnancy >12 weeks' gestation, (2) no symptoms or physical signs consistent with deep venous thrombosis or superficial thrombophlebitis, and (3) no previous history of deep venous thrombosis, superficial thrombophlebitis, or venous insufficiency. After informed consent was obtained, the light-reflection rheography measurements were carried out. A total of 28 patients with uncomplicated pregnancies were ex-

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Table I	Reculte	TATITA	nationte	117	eitting	position
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	Patients	Extremities tested	Diela I	I of low	≤0.31	mm/sec
Group	(No.)	(No.)	Right leg (mm/sec)	Left leg (mm/sec)	No.	%
13-27	11	22	0.57 ± 0.23	0.56 ± 0.21	2	9
28-42	17	30	0.59 ± 0.24	0.48 ± 0.17	1	3
TOTAL	28	52	0.58 ± 0.23	0.52 ± 0.19	3	6

Table II. False-positive results in other positions

Position	False-positive results			
Position	No.	%		
Sitting with a tilt Lateral decubitus	4/11 4/13	36 31		

amined, 11 at 13 to 27 weeks' gestation and 17 between 28 and 42 weeks.

Measurements were taken with the patient seated comfortably in the chair with both feet flat on the floor, as for nonpregnant subjects. Pedal pulses were examined to rule out arterial occlusive disease. The light-reflection rheography sensor head was attached to the leg to be tested 10 cm above the medial malleolus. The patient was instructed to dorsiflex the foot 10 times over 15 seconds. The slope of the venous emptying curve by light-reflection rheography was calculated by the formula:

Slope =
$$\Delta R/T_1$$

where ΔR is venous drainage and T_1 is measured in seconds. The result is the rise of the evacuation curve in millimeters per second (rate of emptying).⁶ A slope of <0.31 mm/sec was used as the value indicating deep venous thrombosis, whereas a normal slope was 0.54 ± 0.06 in nonpregnant subjects.⁵ After the initial examination with the patient in a sitting position, a subset of patients in the third trimester were retested in the lateral decubitus position, the supine position, and a sitting position with a tilt to determine if the results of the tests would be affected by the position of the gravid uterus. A paired t test was used to determine statistical significance. A t value of t0.05 was considered significant.

Results

In the standard sitting position the results were very similar to the previous results in nonpregnant patients. As Table I demonstrates, the average slope in all pregnancies tested was 0.58 ± 0.23 (ISD) for the right leg and 0.52 ± 0.19 (\pm SD) in the left leg (p = 0.21). The false-positive rate with a slope ≤ 0.31 mm/sec was 6% (n = 3).

In a subset of 11 patients in the third trimester other positions were tested to determine if shifting the weight of the uterus from the inferior vena cava would affect the results (Table II). In 11 patients the right leg was tested while a wedge was placed under the right hip to shift the weight to the left side. Four of these patients had abnormal examination results for a false-positive rate of 36%. Thirteen subjects were placed in the semi-Fowler left lateral decubitus position for the same purpose, and four of them had abnormal test results for a false-positive rate of 31%. In the supine position none of the test results revealed a deflection curve at all.

Comment

Acute deep venous thrombosis results in significant increases in morbidity and mortality during pregnancy as a result of pulmonary emboli. Unfortunately, pregnant patients frequently have signs and symptoms consistent with deep venous thrombosis necessitating performance of noninvasive screening tests that have a high false-positive rate and are very operator dependent. Venography is the technique that yields the most accurate information regarding the extent of occlusion and location of thrombosis, but it carries a significant morbidity rate. A noninvasive test for deep venous thrombosis that is sensitive and specific and requires minimal operator skill would be desirable.

Light-reflection rheography appears to fulfill these requirements. It has been shown to be 92% sensitive and 94% specific in previous studies involving non-pregnant patients. ^{5,6} Prior investigations have also demonstrated that false-positive results may occur in elderly patients and subjects with a history of congestive heart failure or leg edema. ⁵ The technique has not been tested in pregnant women. It seems possible that the gravid uterus might affect venous return from the lower extremities and thus increase the false-positive rate of this test.

Before a prospective study comparing venography with light-reflection rheography during pregnancy was begun, it was necessary to determine whether the gravid uterus would obstruct the venous outflow from the lower extremities frequently enough to make light-reflection rheography ineffective in the assessment of patients with a suspected deep venous thrombosis. The standard sitting position that has been used in previous

studies was also the most effective among the pregnant patients in this investigation. Other positions tested did not produce reliable results. The finding of no deflection on light-reflection rheography in the supine position was not surprising in that there is not a column of blood to be affected by gravity as in the other positions. The amount of time required to train our personnel to perform the test was minimal (<30 minutes' instruction and practice on two patients). The pregnant patients were easily able to dorsiflex the foot as instructed. These results demonstrate a false-positive rate of only 6%, which is consistent with the 94% specificity previously reported in nonpregnant patients.5

This preliminary study demonstrates that the gravid uterus does not appear to affect the lower-extremity venous emptying time enough to cause an unacceptable false-positive rate. Since this is an easily performed, noninvasive test, a prospective study comparing lightreflection rheography with venography and other noninvasive tests during pregnancy is currently under way.

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Anticardiolipin antibody—positive serum enhances endothelial cell platelet-activating factor production

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Circulating antiphospholipids have been linked to recurrent pregnancy loss by a mechanism involving placental and decidual thrombosis. We hypothesized that platelet-activating factor, an autacoid synthesized by vascular endothelium, might mediate this phenomenon through its ability to promote platelet aggregation and fibrin deposition. Alternatively, antiphospholipid antibodies might exert a procoagulant effect by inhibiting the synthesis of prostacyclin. To evaluate these theories, endothelial cells (harvested from human umbilical veins) were grown to confluence and incubated for 48 hours with 20% concentrations of anticardiolipin antibody-positive and -negative human sera as well as fetal bovine serum. After incubation culture wells were stimulated with 10 µmol/ml calcium ionophore A23187 (an agonist of platelet-activating factor and prostacyclin synthesis). Intracellular platelet-activating factor was measured by tritiated acetate incorporation, phospholipid extraction, thin-layer chromatography, and scintillation spectrophotometry. Enhanced platelet-activating factor synthesis was identified in cultures incubated with anticardiolipin antibody-positive serum (25,544 ± 2604 disintegrations per minute, mean ± SD) when compared with anticardiolipin antibody-negative serum (18,600 ± 3316 dpm) or fetal bovine serum (19,014 \pm 4233 dpm; analysis of variance, p = 0.033). In similar experiments, prostacyclin synthesis was determined by measuring its primary metabolite, 6-keto-prostaglandin F_{1a}, in culture supernatants. No differences between anticardiolipin antibody-positive and control cultures were observed (analysis of variance, p = 0.90). We conclude that in this endothelial cell model, anticardiolipin antibody-positive serum enhances ionophore-mediated platelet-activating factor synthesis but has no apparent effect on the production of prostacyclin. These findings suggest a potential role for platelet-activating factor in anticardiolipin antibody-mediated vascular thrombosis. (AM J OBSTET GYNECOL 1991;165:1748-52.)

Key words: Anticardiolipin antibody, platelet-activating factor, endothelial cell

Anticardiolipin antibody is among the family of circulating antiphospholipids that have been linked to adverse pregnancy outcome. It is postulated that the continuum represented by recurrent miscarriage, suboptimal fetal growth, and stillbirth is really a graded expression of a common pathophysiology, namely antibody-mediated intravascular thrombosis at the maternal-trophoblast interface. Normally the hemostatic mechanisms favoring anticoagulation predominate so that blood flow remains relatively unimpeded and thrombus formation does not occur. Antiphospholipid antibodies are thought to perturb this equilibrium, favoring thrombosis by a mechanism(s) as yet undefined.

Previous investigations have focused on the possibil-

ity that antiphospholipid antibodies decrease the concentration or potency of anticoagulant factors such as protein C, antithrombin III, or prostacyclin. ⁴⁻⁶ Observations regarding these anticoagulants have been at odds ^{6, 7} or inconclusive. ⁸ The alternative that selected procoagulant factors may be enhanced by anticardiolipin antibody has not been fully evaluated. Platelet-activating factor is known to stimulate a wide variety of biologic responses, many of which are procoagulant in effect. ^{9, 10} The ability of vascular endothelium ^{11, 12} to synthesize platelet-activating factor makes it an appealing mediator to study in the context of anticardiolipin antibody—mediated thrombosis.

Using primary cultures of human umbilical vein endothelial cells, we evaluated the hypothesis that anticardiolipin antibody—positive serum enhances the synthesis of platelet-activating factor. We also explored the possibility that production of the anticoagulant prostacyclin might be inhibited in the presence of anticardiolipin antibody. If demonstrated, these two biochemical alterations would support the presumed link between anticardiolipin antibody and altered hemostasis.

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Table I. Selected characteristics of anticardiolipin antibody—positive serum donors

, Patient	Highest observed anticardiolipin antibody (IgG) in patient (GPL units/ml)	Current anticardiolipin antibody (IgG) for serum used in experiments (GPL units/ml)	Obstetric history
A	131	131	SAB × 2; 120 gm, 23 wk IUFD
В	10	9	$SAB \times 3$
C	2800	340	Nulliparous with scleroderma
D	14 (MPL/ml)	12 (MPL/ml)	560 gm, 26 wk neonatal death
E	24	20	SAB × 2; 770 gm, 28 wk; SLE; infant survived
F	75	75	$SAB \times 5$; 16 wk IUFD; SLE
G	10	10	SAB × 2; 630 gm, 31 wk; infant survived

GPL units correspond to the binding activity of 1 μg/ml of purified anticardiolipin antibody. Normal values: IgG <3 GPL/ml; IgM <5 MPL/ml (SmithKline Bioscience). SAB, Spontaneous abortion; IUFD, intrauterine fetal death; SLE, systemic lupus ery-

Material and methods

Collection of serum samples. Blood was collected from seven volunteers with documented anticardiolipin antibody whose obstetric histories were consistent with antibody-mediated reproductive failure (Table I). Those with overt autoimmune disease were not receiving medication at the time of venipuncture. Samples were also obtained from seven control subjects (anticardiolipin antibody-negative without prior pregnancy loss). All sera were heat inactivated (56° C for 30 minutes), frozen (-70° C), and coded to blind the laboratory personnel. An aliquot of each sample was sent to a laboratory (SmithKline Bioscience, Van Nuys, Calif.), where enzyme-linked assays for each immunoglobulin (Ig) subclass (G, M, and A) were performed.

Endothelial cell cultures. Fresh umbilical cords (without meconium staining, identified from uncomplicated term pregnancies) were placed in sterile saline solution and refrigerated (<24 hr) until processing by the methods of Prescott et al.11 The veins were cannulated, rinsed with Hanks' balanced salt solution, and incubated with collagenase (0.1% in Hanks' balanced salt solution; Wurthington Biochemical Co., Freehold, N.J.) for 15 minutes at 37° C. The venous effluents from all cords were combined to ensure that all wells in a given experiment had an equal distribution of cells from each cord. After centrifugation (1100 rpm for 10 minutes at 25° C), the cell pellets were suspended in complete medium 199; 20% fetal bovine serum, penicillin and streptomycin (5000 units/ml each), and gentamicin (50 ng/ml), and then inoculated into 12-well culture dishes. Monolayer confluence was usually achieved in 4 to 7 days (in 100% humidity, 5% carbon dioxide at 37° C), with replacement of growth medium (minus gentamicin) at 48-hour intervals. Confirmation of cell type was accomplished by morphologic evaluation under phase contrast microscopy and by identification of factor VIII antigen. Preexperimental and postexperimental viability testing was assured by trypan blue exclusion.

Experimental design. Confluent monolayers were randomly assigned for exposure to either anticardiolipin antibody-positive or anticardiolipin antibody-negative serum, or fetal bovine serum. The serum concentrations (10% to 40%) and duration of incubation (range 1 to 48 hours) were intentionally varied during preliminary experiments. The agonist calcium ionophore (A23187) was used in all experiments because unstimulated monolayers synthesized negligible amounts of platelet-activating factor. All experiments were performed in triplicate with an individual anticardiolipin antibody-positive and anticardiolipin antibody-negative serum source. Statistical analysis was by analysis of variance and linear regression (least squares), with significance at p < 0.05.

Quantification of platelet-activating factor. Intracellular platelet-activating factor was measured according to the methods of Zimmerman et al.12 After incubation with protocol sera, monolayers were washed and then simultaneously treated with modified Hanks' balanced salt solution (containing Ca++ and Mg++), ionophore A23187, and carrier-free tritiated acetate (25μCi/ml). After a 15-minute incubation at room temperature, the reaction was stopped with the addition of 50 mmol/L acetic acid in methanol. The lipids were extracted in chloroform (15 minutes, 4° C centrifugation), dried under nitrogen, and resuspended in chloroform/methanol (9:1). Twenty-five microliters of sample solution was removed and reserved as the direct fraction; the remaining lipids were applied to silica gel plates for separation by thin-layer chromatography. Areas containing platelet-activating factor (corresponding to the synthetic platelet-activating factor standard) and the remaining lipids were scraped into separate scintillation vials for spectrophotometry. Platelet-activating factor was calculated as a fraction of the total extracted lipids and expressed as disintegrations per minute.

Quantification of 6-keto-prostaglandin Fig. Experiments parallel to those described for comparison of

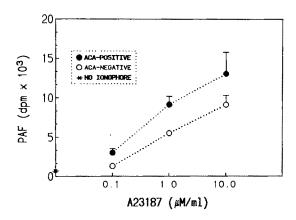


Fig. 1. Effect of increasing dosages of calcium ionophore A23187 on platelet-activating factor (PAF) synthesis from confluent endothelial monolayers after preincubation with anticardiolipin antibody (ACA)-positive and -negative serum. Anticardiolipin antibody-negative serum: $r^2 = 0.99$; p = 0.018. Anticardiolipin antibody-positive serum: $r^2 = 0.98$; p = 0.081.

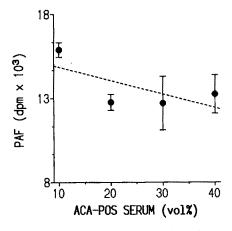


Fig. 2. Platelet-activating factor (PAF) synthesis from confluent endothelial monolayers was not affected by increasing concentrations of anticardiolipin antibody—positive (ACA-POS) serum. *Dotted line*, Linear regression $(r^2 = 0.47; p = 0.31)$.

platelet-activating factor synthesis between anticardiolipin antibody—positive, anticardiolipin antibody—negative, and fetal bovine sera were also performed to evaluate differences in prostacyclin synthesis and release. After stimulation with calcium ionophore A23187, all culture supernatants were aspirated and frozen (-70° C). A standard radioimmunoassay (Advanced Magnetics, Cambridge, Mass.) was used to quantitate this eicosanoid, which was expressed an nanograms per milliliter.

Results

Dosage of calcium ionophore. To confirm a doseresponse relationship in this model, we varied the agonist concentration and measured the corresponding

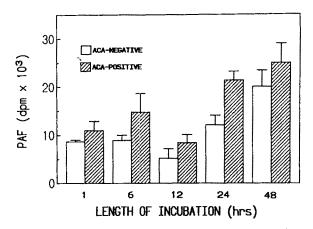


Fig. 3. Effect of varying the duration of serum exposure on platelet-activating factor (*PAF*) synthesis from confluent endothelial monolayers after incubation with anticardiolipin antibody (*ACA*)—positive and —negative serum.

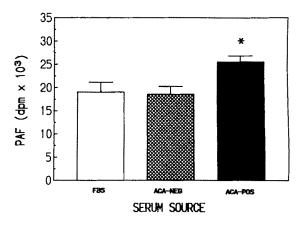


Fig. 4. Platelet-activating factor (*PAF*) synthesis from confluent endothelial monolayers after incubation for 48 hours with anticardiolipin antibody—positive (*ACA-POS*), anticardiolipin antibody—negative (*ACA-NEG*) serum and fetal bovine serum (*FBS*), all at 20% concentration. The agonist was 10 μ mol/ml A23187. Platelet-activating factor synthesis in anticardiolipin antibody—positive serum was significantly greater than in both control groups (analysis of variance, F = 5.1; p = 0.033).

platelet-activating factor synthesis. (Fig. 1; anticardiolipin antibody—positive serum from patient E, Table I). Very little platelet-activating factor was recovered in the absence of ionophore stimulation (698 \pm 79 dpm, mean \pm SD) and a dose response was observed with increasing ionophore concentration in cultures incubated with anticardiolipin antibody—positive and control sera. In subsequent experiments we chose to use $10 \,\mu$ mol/ml of the ionophore because that dose seemed to provide the most consistent response (e.g., in the range of 10^4 dpm).

Serum concentration and length of incubation. Increasing the concentration of anticardiolipin antibody—positive serum (from 10% to 40%) did not significantly

Table II. Comparison of platelet-activating factor synthesis among confluent monolayers incubated with 20% anticardiolipin antibody-positive, anticardiolipin antibody-negative, and fetal bovine serum for 48

Patient	Anticardiolipin antibody positive (dpm, mean ± SEM)	Anticardiolipin antibody negative (dpm, mean ± SEM)	Fetal bovine serum (dpm, mean ± SEM)
С	$27,302 \pm 4930$	$13,638 \pm 511$	19,904 ± 3107
D	$24,941 \pm 2337$	$20,030 \pm 1932$	$16,047 \pm 62$
F	$27,816 \pm 2200$	$20,574 \pm 1539$	$24,636 \pm 2342$
G	$22,119 \pm 1227$	$20,160 \pm 775$	$15,471 \pm 674$

Ionophore-negative culture wells used to assess unstimulated platelet-activating factor synthesis (data not shown). All other wells indicated for 15 minutes with 10 μmol/ml ionophore A23187 during tritium acetate incorporation. Data represent mean value of three experimental wells.

alter platelet-activating factor synthesis (Fig. 2; anticardiolipin antibody-positive serum from patient B, Table I). In subsequent experiments a 20% serum concentration was used.

The length of incubation appeared to influence the degree of platelet-activating factor synthesis in response to ionophore exposure (Fig. 3). The data in Fig. 3 represent two separate experiments (1, 6, and 12 hours and 24 and 48 hours) with the same anticardiolipin antibody serum source (patient A, Table I). We chose an incubation length of 48 hours for additional experiments but continued to evaluate shorter intervals if sufficient culture wells and serum were available.

Platelet-activating factor and prostacyclin production. The effect of anticardiolipin antibody-positive serum (from patients C, D, F, and G) on both plateletactivating factor and 6-keto-prostaglandin Fia synthesis was compared with the effect of control serum (anticardiolipin antibody-negative and fetal bovine serum); the raw data on intracellular platelet-activating factor recovery are shown in Table II. A significant increase in platelet-activating factor was found in monolayers incubated with all four anticardiolipin antibody-positive sera tested (Fig. 4; analysis of variance, p = 0.033). Post hoc analysis (Newman-Keuls test) revealed a significant increase between anticardiolipin antibodypositive serum and both control groups. In duplicate experiments, 6-keto-prostaglandin F1a was recovered from the culture media and quantified. No differences between anticardiolipin antibody-positive and control cultures were observed (anticardiolipin antibody-positive, 69 ± 29 ng/ml, mean \pm SD; anticardiolipin antibody-negative: 63 ± 31 , mean \pm SD; fetal bovine serum, 65 ± 23 , mean \pm SD; analysis of variance, p = 0.90).

Comment

Platelet-activating factor is an exceedingly potent lipid mediator with diverse biologic activities in health and disease. In addition to influencing aggregation and degranulation, platelet-activating factor directly influences the synthetic activity of many cell types, including neutrophils, macrophages, lymphocytes, and hepatocytes.9 Although platelet-activating factor has been proposed to participate in a variety of disease states, including diabetes, asthma, cirrhosis, and endotoxic shock, the links to connective tissue disease and to vascular thrombosis suggest a possible role for plateletactivating factor in antibody-mediated reproductive failure.

We consistently observed an enhancement of plateletactivating factor synthesis in the presence of anticardiolipin antibody-positive sera from a variety of women with adverse reproductive and medical histories. We cannot determine whether the antibody itself was the cause or if another serum factor(s) unique to these women might have promoted excess platelet-activating factor synthesis. Although we did not evaluate antibody-endothelial cell binding in our cultures, its occurrence has been demonstrated by Schorer et al.6 using similar methods. For the present, we assume that an antibody-endothelial cell interaction may contribute to anticardiolipin antibody-mediated pathophysiologic conditions, while recognizing that other cellular targets (e.g., platelets) or soluble cofactors may be involved as well.

Because a majority of newly synthesized platelet-activating factor remains stored within the endothelial cell,13 the question arises as to how its procoagulant effect is exerted within the vascular space. It has been demonstrated that platelet-activating factor is incorporated into the cell membrane,10 thus providing the opportunity to exert its chemotactic properties on platelets and other cells within the vasculature. Once platelet binding takes place, the cascade leading to thrombosis might begin. Alternatively it is possible that after prolonged endothelial cell exposure to anticardiolipin antibody the excess intracellular platelet-activating factor is only released subsequent to endothelial cell damage by a secondary mechanism. Because of its potency and its ability to promote additional eicosanoid synthesis (through arachidonic acid release from surrounding phospholipids¹⁴), a relatively small quantity of liberated platelet-activating factor may be sufficient to trigger thrombogenesis. In this regard it is also notable that the activity of platelet-activating factor's primary catabolic enzyme, acetylhydrolase, is greatly diminished during pregnancy,¹⁵ a factor that might amplify the impact of even a physiologic concentration of this autacoid. Implicating platelet-activating factor directly in the pathophysiology of the antiphospholipidantibody syndrome may ultimately have clinical ramifications because there are already specific diseases (e.g., asthma) for which clinical trials of platelet-activating factor—receptor antagonists are being evaluated.

Future studies should attempt to clarify whether the autoantibodies themselves or other factors are responsible for the enhanced platelet-activating factor synthesis observed in these experiments. Additionally, evaluation of endothelial cells from the umbilical veins of anticardiolipin antibody—positive pregnancies may confirm antibody binding and may also identify excess intracellular platelet-activating factor levels resulting from chronic in vivo antibody exposure.

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Combination antibiotics and indomethacin in idiopathic preterm labor: A randomized double-blind clinical trial

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Subclinical infection may play a role in the failure of magnesium sulfate tocolysis. Using a double-blind randomized study design, we administered a combination of ampicillin-sulbactam and indomethacin or corresponding placebos to patients in preterm labor who were receiving intravenous magnesium sulfate tocolysis. The mean gestational age at enrollment was 30.1 weeks, and mean cervical dilatation was 2.15 cm. No differences were noted between placebo (n=43) and study patients (n=43) in gestational age at delivery, term deliveries, days gained, or neonatal outcome. Preterm delivery (<36 weeks) occurred in 61% of the total population. The likelihood of a β error was 0.07 to 0.23 on the basis of outcome analysis. In our population adjunctive ampicillin-sulbactam with indomethacin did not improve the success of magnesium sulfate tocolysis. (AM J OBSTET GYNECOL 1991;165:1753-9.)

Key words: Preterm labor, antibiotics, indomethacin

The majority of neonatal deaths, morbidity, and perinatal health care costs are associated with birth at <36 weeks. The ability of tocolytics to prevent preterm birth varies by site and study design rather than agent. In comparative trials the likelihood of tocolytic failure ranges from 28% to 81% for ritodrine, ¹⁻⁵ 39% to 61% for magnesium sulfate, ³⁻⁷ and 20% to 55% for terbutaline. ^{2-1,8} These high failure rates underline the need for better tocolytic regimens.

In the past 15 years, a wide body of epidemiologic, biochemical, and clinical evidence has supported the role of subclinical infection in preterm birth. Bacterial species differ in the strength of their association with preterm birth. However, genital tract anaerobes have been associated consistently with preterm birth by different authors and different institutions. If subclinical infection plays a role in preterm birth, then antibiotic therapy that eradicates anaerobes should prevent preterm birth in that subgroup of patients.

Tocolytic failure is associated with positive amniotic fluid cultures or an elevated C-reactive protein level. Ten to twenty percent of amniotic fluid cultures are positive in patients with idiopathic preterm labor. 14-17 With the exception of Duff and Kopelman, 16 most authors have associated positive amniotic fluid cultures with tocolytic failure. Potkul et al. 18 showed that 23 of

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24 patients (96%) with normal C-reactive protein levels had successful tocolysis, whereas 10 of 16 patients (62%) with elevated C-reactive protein levels had tocolytic failure. Similarly, Handwerker et al.¹⁹ showed that 33 of 35 (94%) had tocolytic success with normal C-reactive protein levels and 11 of 15 (73%) had tocolytic failure with elevated C-reactive protein levels. Given these observations, the addition of antibiotic therapy to standard tocolytic therapy may reduce the likelihood of tocolytic failure.

The use of antibiotics has a potential to increase uterine activity transiently. The lysis of bacteria releases endotoxin from the cell walls. Subsequently, endotoxin will stimulate the macrophages to release cytokines or enzymes, which initiates prostaglandin synthesis.²⁰ As macrophage-like cells are numerous in the decidua, the surge of endotoxin-induced prostaglandins may counteract the benefits of adjunctive antibiotics. Therefore a short course of a prostaglandin inhibitor may enhance the benefits of adjunctive antibiotic therapy.

McGregor et al.²¹ in 1986 reported the first controlled trial of antibiotics in patients with active preterm labor. With subsequent support by Morales et al.²² and Winkler et al.²³ in 1988, adjunctive antibiotics seemed to reduce the incidence of preterm birth in a subgroup of patients with cervical dilatation ≥1 cm. In contrast to the latter studies, we reported in 1989²⁴ that the combination of ampicillin plus erythromycin used in conjunction with standard tocolytics failed to improve outcome.

If we assume subclinical infection to be a major cause of tocolytic failure, our discrepant results have been due to differences in study design, population differences in genital tract flora, inadequate anaerobic coverage, or an increase in uterine activity that was precipitated by a surge of endotoxin-induced prostaglandins from the lysis of bacteria. In the current study we examined the last two possibilities. Our study hypothesis was that the combination of antibiotics with anaerobic coverage (ampicillin-sulbactam) with a prostaglandin inhibitor (indomethacin) will reduce the incidence of tocolytic (magnesium sulfate) failure.

Material and methods

Subjects were selected from hospitalized patients at Medical Center Hospital in San Antonio. The Medical Center serves an indigent, predominantly Mexican-American population, who are cared for by the residents and full-time faculty at The University of Texas Health Science Center at San Antonio. The protocol was approved by the Institutional Review Board of the Health Science Center.

We used the following criteria to select eligible patients: (1) preterm gestation between 24 weeks and 33 weeks 6 days or an estimated fetal weight between 750 and 2200 gm by ultrasonographic measurement and (2) premature labor diagnosed by three contractions in 20 minutes and observed cervical effacement or dilatation or cervical dilatation of ≥2 cm and 50% effacement at initial examination.

Upper-level residents in obstetrics and gynecology, under the supervision of maternal-fetal medicine faculty, made the diagnosis of preterm labor, on the basis of cervical examination and contraction monitoring. Gestational age was determined from menstrual dating, date of pregnancy test, serial fundal height measurement, and previous obstetric ultrasonographic examination. In addition, every patient had a detailed ultrasonographic evaluation of the fetus on admission. When menstrual data were considered unreliable, a mean gestational age was determined by biparietal diameter, head circumference, femur length, and abdominal circumference. The exclusion criteria were premature rupture of membranes, suspected intrauterine growth retardation (estimated fetal weight < 10th percentile for gestational age), hypertension, known uterine anomalies, incompetent cervix, third-trimester bleeding, oligohydramnios, abnormal fetal testing, liver disease, asthma, known allergies to indomethacin or ampicillin-sulbactam, or clinical evidence of maternal infection.

The study design is depicted in Fig. 1. Tocolytic therapy was the same for both groups. We chose magnesium sulfate because it is our standard tocolytic. Preterm patients with at least three contractions in 20 minutes, with or without cervical dilatation, were hospitalized and received hydration and sedation. Only patients with contractions in spite of the latter therapy were given parenteral intravenous magnesium sulfate: a

4 gm loading dose followed by 2 gm/hr and adjusted every 30 minutes to maintain uterine quiescence (fewer than one contraction in 15 minutes) or a magnesium sulfate level of 6 to 8 mEq/dl. Of these, only patients with a cervical change or cervical dilatation \geq 2 cm and \geq 50% effacement who met selection criteria were enrolled.

Before the administration of study medications, we obtained blood for serum chemistry tests, complete and differential blood cell counts, C-reactive protein level, and an erythrocyte sedimentation rate. Vaginal Gram's stain was performed to detect bacterial vaginosis (bacterial vaginosis score ≥ 7). Vaginal cultures for aerobes and genital mycoplasmas were performed, and organisms were isolated and identified with standard techniques. A cervical sample was obtained for cultures (Neisseria gonorrhoeae) and an antigen test for Chlamydia trachomatis (Microtrak) was performed. Patients in whom group B streptococcus or C. trachomatis were identified were not treated until delivery was unavoidable.

The study medications were assigned randomly in a 1:1 ratio by the pharmacy and administered in a double-blind fashion. Patients received intravenous ampicillin (2 gm)—sulbactam (1 gm) every 6 hours for 12 doses plus concomitant oral indomethacin (50 mg loading dose, followed by 25 mg every 6 hours for seven doses) or corresponding placebos.

Indomethacin was chosen as a prostaglandin synthetase inhibitor because of its clinical experience in obstetrics in the treatment of preterm labor and the lack of neonatal effects when it is used as a tocolytic. Fetal echocardiography was performed on each fetus at 24 and 48 hours after enrollment to monitor changes in cardiac dimensions that may be related to premature closure of the ductus arteriosus. The results of the cardiac biometry were not reported to the clinicians and did not change clinical management. The findings are the subject of a separate report.

After successful therapy with parenteral tocolytics patients were treated with oral magnesium oxide (400 mg every 3 to 4 hours) or oral terbutaline sulfate (2.5 to 5 mg every 3 to 4 hours). The patients were hospitalized as long as was clinically appropriate, usually 3 to 4 days. Undelivered patients had vaginal cultures and vaginal Gram's stain repeated between 2 and 14 days after therapy.

If preterm labor necessitating intravenous magnesium sulfate recurred and in those patients whose contractions did not stop with a serum magnesium level of 6 to 8 mEq/dl, amniotic fluid was obtained by amniocentesis for Gram's stain, aerobic and anaerobic culture, and fetal lung maturity studies. If the amniotic fluid Gram's stain demonstrated bacteria or fetal lung maturity study results were positive, tocolysis was discon-

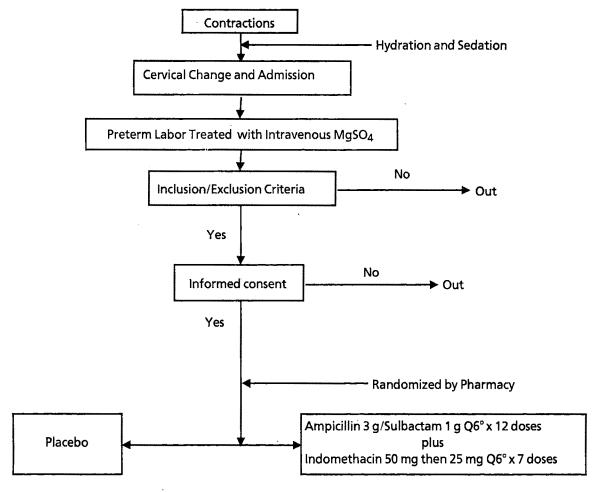


Fig. 1. Flow chart.

tinued. If the amniotic fluid study results were negative, tocolytic therapy was changed to ritodrine hydrochloride. Tocolytic failure was defined as labor with cervical dilation ≥6 cm, clinical infection, rupture of the membranes, or fetal distress and subsequent preterm birth.

Birth weight and gestational age at delivery were considered the most important outcome variables. A sample size was determined with these variables. Clinically significant increases in mean birth weight and gestational age at delivery were estimated to be 250 gm and 7 days, respectively. The variances in birth weight and gestational age at delivery were estimated to be 500 gm and 14 days, respectively. Assuming an α-error threshold of 0.05 and a power of 0.8, we determined that a sample size of 49 in each arm would be sufficient to determine significance.

Univariate analyses were performed with the Student t test and analysis of variance for continuous data. Nonparametric tests (Mann-Whitney U test) were used to confirm the validity of parametric testing and to determine the probability of differences between the groups in variables with nonnormal distributions. Cat-

egoric data were analyzed with χ^2 and Fisher's exact tests. Product limit survival analysis26 was used to describe differences in days gained in placebo and study groups. Probabilities <0.05 were considered significant.

Results

Between July 1989 and January 1991, we identified 298 laboring women who met the gestational age criteria (24 weeks to 33 weeks 6 days), who had cervical change or cervical dilatation >2 cm and cervical effacement >50% and who were being treated with parenteral magnesium sulfate. Thirty-four (14%) met the criteria but were delivered before informed consent was obtained. Most of these patients were admitted at cervical dilatations of 4 to 5 cm and were delivered shortly thereafter. Thirty-seven (15%) eligible patients either refused participation or were not identified. The remaining 88 (35%) met the exclusion criteria, including 21 (8%) with abnormal fetal test results, 15 (6%) with third-trimester bleeding, and 13 (5%) with concurrent antibiotic use. The mean birth weight of those

Table I. Population characteristics at enrollment

. Parameter	$Placebo \\ (n = 43)$	Ampicillin-sulbactam plus indomethacim $(n = 43)$
Age (yr, mean ± SD)	23.8 ± 5.0	23.8 ± 5.5
Nulliparity (No.)	16	11
Gestational age (wk, mean ± SD)	30.3 ± 5.0	29.8 ± 2.7
Estimated fetal weight (gm, mean ± SD)	1601 ± 422	1624 ± 533
Cervix (cm, mean ± SD)	2.13 ± 0.93	2.1 ± 0.95
Elevated C-reactive protein level (No.)	28	24
White blood cell count ≥12,000 cells/mm³ (No.)	31	. 28

Table II. Microbiologic composition of vagina

		Placebo (n=43		Ampic	illin-sulbacta (n =	m plus indor = 43)	nethacin
		Enrollment Follow-up $(n = 45)$ $(n = 15)$		Enrollment $(n = 43)$		Follow-up (n = 15)		
Organism	No.	% .	No.	%	No.	. %	No.	%
Aerobic gram-negative rods	11	24	6	40	11	24	9	60*
Mycoplasma hominis	16	36	5	33	16	37	2	13
Ureaplasma urealyticum	24	53	5	33	20	46	6	40
Gardnerella vaginalis	28	62	8	53	12		2	13*
Group B streptococcus	. 2	4	2	13	7	16	0	
Lactobacillus sp.	21	47	5	33	27	63	2	13*
N. gonorrhoeae	0		0		0		0	
C. trachomatis	5		0		4		4	
Bacterial vaginosis	17	38†	6	40	7	16	1	7

^{*}Change in vaginal flora at follow-up, p < 0.05.

not included in the study was 1620 ± 749 gm. Ninety-one patients (31%) met the selection criteria and were enrolled. Subsequent to enrollment, five patients were dropped from analysis, one because of delivery before study drug administration, one as a result of concurrent antibiotic therapy for a urinary tract infection at entry, and three patients who had successful tocolysis but were lost to follow-up after discharge and before 36 weeks. Forty-three placebo and 43 study drug patients were used in the analysis of outcome data. The enrollment was halted early (91 enrolled vs 98 projected patients) for administrative reasons.

Tables I and II describe the population characteristics at enrollment. Our selection criteria and randomization process created similar populations in the placebo and study drug groups except for the presence of bacterial vaginosis. The presence of more bacterial vaginosis in the placebo group than in the treatment group (38% vs 16%, p < 0.05) would bias against the placebo group and decrease an antibiotic effect. Antibiotics increased the likelihood of aerobic gram-negative rods, decreased *Lactobacillus* sp. and *G. vaginalis* at follow-up. Twelve (14%) patients underwent amniocentesis; none had an intraamniotic infection. The median times from admission to administration of study

drugs or placebo were 8 and 7 hours, respectively. This delay was not correlated with birth weight (r = 0.1, p, not significant).

Table III describes maternal outcome. There were no differences in gestational age at delivery, deliveries at >35 weeks, days gained from enrollment in the study, number of patients with recurrent preterm labor, or maternal infection (intraamniotic infection or endometritis). Fig. 2 describes the cumulative deliveries by weeks after enrollment. The curves are similar visually and statistically. Of note, 80% in both groups were undelivered at 1 week after enrollment.

Table IV depicts the neonatal outcome. Six sets of twins were enrolled (two placebo, four study drug). As a result, neonatal outcome was analyzed in 45 placebo and 47 study drug infants. There was no difference in perinatal mortality and morbidity. The net gain in fetal weight (birth weight minus estimated fetal weight) was 789 ± 674 gm in study drug patients and 645 ± 649 gm in placebo patients (p = not significant). One case of group B streptococcal disease was noted in a 2330 gm infant who was delivered 25 days after enrollment. The mother received placebo, and the culture for group B streptococcus was negative at enrollment. The infant did well. Another neonate, weighing 694 gm,

[†]Placebo versus antibiotic group at enrollment, p < 0.05.

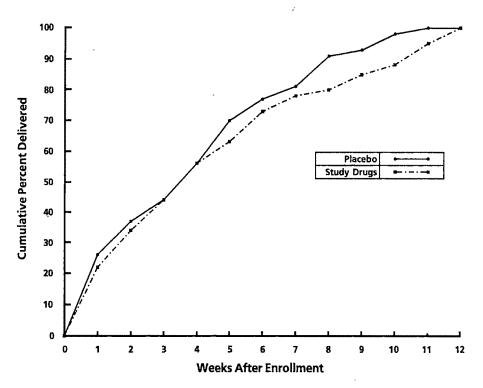


Fig. 2. Graph of days gained.

Table III. Maternal outcome

Parameter	$\begin{array}{l} Placebo\\ (n=43) \end{array}$	Ampicillin-sulbactam plus indomethacin $(n = 43)$
Gestational age (wk, mean ± SD)	33.5 ± 4.0	34.5 ± 4.1
Delivery ≥36 wk		
No.	16	20
%	37	46
Days gained		
Median	26	26
Range	1-71	1-79
Recurrent preterm labor (No.)	8	8
Maternal infection (No.)	6	1

was born on the third day of ampicillin-sulbactam plus indomethacin therapy. On the seventh day of life, the neonate had a clinical course consisting of sepsis and death but cultures were negative. The other perinatal death was an intrapartum death of a 750 gm, previable (25-week) fetus on the third day of ampicillin-sulbactam plus indomethacin therapy. Chorioamnionitis developed, and the fetus died during a complicated breech extraction. No autopsy was performed.

We stratified the population to identify a subgroup where antibiotics plus prostaglandin inhibitors might be of benefit. No improvement in outcome was noted in singleton gestations (n = 82), in patients at <31weeks (n = 35), elevated C-reactive protein levels (n = 52), enrollment white blood cell count >12,000 cells/mm 3 (n = 59), abnormal vaginal flora (positive group B streptococcus or bacterial vaginosis) (n = 33), or the presence of *U. urealyticum* at enrollment (n = 44). However, the likelihood of a β error as a result of small numbers is as high as 60%.

Comment

The addition of ampicillin-sulbactam plus indomethacin to magnesium sulfate tocolysis did not reduce the likelihood of preterm birth subsequent to tocolytic failure in our population. The validity of our conclusion was supported by selection criteria that identified a population where 60% were delivered at <36 weeks and <2500 gm, a randomization process that created similar populations in both treatment arms (except for the presence of bacterial vaginosis) and the probability of a β error, the likelihood of missing a true difference, of 0.23 on the basis of number of term deliveries, 0.19 on the basis of birth weight, and 0.07 on the basis of number of infants without perinatal morbidity.

The findings are consistent with our prevous study.24

Table IV. Neonatal outcome

Parameter	$Placebo \\ (n = 45)$	Ampicillin-sulbactam plus indomethacin $(n = 47)$
Birth weight <2500 gm (gm, mean ± SD)	2295 ± 795	2430 ± 899
No.	31	26
%	69	55
Hyaline membrane disease (No.)	13	12
Mechanical ventilation	11	12
Intraventricular hemorrhage (No.)	2	2
Sepsis (No.)	1	1
Necrotizing enterocolitis (No.)	1	1
5 min Apgar score ≤7 (No.)	5	4
Perinatal death (No.)	0	2
Congenital abnormalities (No.)	0	2
No perinatal morbidity		
No.	26	29
%	58	62

Patients enrolled in the current study were at higher risk for preterm birth than were those in the former study. The differences included cervical dilatation at entry (2.15 vs 2.0 cm, p < 0.05), gestational age at enrollment (30.0 vs 31.7 weeks, p < 0.01), and estimated fetal weight at enrollment (1620 vs 1910 gm, p < 0.01). However, analysis of time gained (26 vs 34 days, p < 0.05), gestational age at delivery (34 vs 36.8 weeks; p < 0.01), and birth weight (2350 vs 2850 gm, p < 0.01) indicated more adverse outcomes in the current study.

Our results are in contrast to those of studies reported by McGregor et al.,²¹ Winkler et al.,²³ and Morales et al.²² Our studies differ in analysis when compared with the latter three studies, which reported beneficial results only in subgroups selected after randomization and during analysis. McGregor et al.²¹ randomized 58 patients and analyzed 17 patients, Winkler et al.²³ randomized 40 patients and analyzed 19, and Morales et al.²² randomized 205 patients and analyzed 150 (three treatment arms). It is possible that their subgroup selection influenced the results.

Differences in population characteristics, especially the presence of vaginal pathogens, may explain our discrepant results. The frequency of positive group B streptococcus (10%) and C. trachomatis (5%) was significantly lower in our population than in that reported by Morales et al.²² (group B streptococcus 21% and C. trachomatis 24%). However, the presence of indirect measures of decidual or parenchymal infection. i.e., elevated G-reactive protein level of elevated white blood cell count, did not improve the results of antibiotic therapy in either of our studies. Perhaps a more specific measure of subclinical decidual infection, i.e., oncofetal fibronectin, may select a population that would benefit from antibiotic therapy.

It is possible that a different antibiotic combination

or dosing schedule might improve outcome. The duration of antibiotic therapy is arbitrary. Single-dose oral therapy successfully eradicates many urogenital infections. The improvement in efficacy of ≥4 days of intravenous therapy over 3 days has not been shown. Our previous study did not show benefit with erythromycin for 7 days. In addition, ampicillin-sulbactam had a biologic affect on vaginal flora (Table II). In fact, the emergence of aerobic gram-negative rods after treatment, in spite of in vitro activity of ampicillin-sulbactam against aerobic gram-negative rods, raises the potential for clinical infection with resistant organisms.

Amniocentesis was not performed before enrollment. When it was used, i.e., for recurrent or persistent preterm labor, the amniotic fluid was not infected, a result consistent with the findings of Duff and Kopelman. ¹⁶ However, amniotic fluid infection is only an indirect measure of decidual infection, and significant decidual infection may be present with negative amniotic fluid cultures. Perhaps measures of amniotic fluid cytokines may be more predictive of decidual infection.

Although >60% of our patients were delivered at <36 weeks, magnesium sulfate tocolysis with or without ampicillin-sulbactam plus indomethacin delayed delivery a median of 26 days. A delay in delivery decreases neonatal morbidity and mortality. Delay in delivery by 1 to 2 weeks in 80% of patients has been observed consistently in controlled studies of magnesium tocolysis³⁻⁷ and was observed in the current study. However, preterm delivery after successful magnesium sulfate tocolysis remains a perplexing clinical problem.

The reasons for failure after successful magnesium sulfate tocolysis are not well described. Brustman et al.²⁷ demonstrated that patients who are delivered prematurely have more frequent contractions in spite of doseadjusted oral tocolytic therapy. Improved agents or de-

livery mechanisms may benefit this high-risk group. The role of recurrent or episodic subclinical decidual infection in this setting remains undefined.

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The progesterone antagonist onapristone increases the effectiveness of oxytocin to produce delivery without changing the myometrial oxytocin receptor concentrations

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The progesterone antagonist onapristone was used in guinea pigs during late pregnancy (43 ± 2 days after coitus) and before term (day 61 after coitus) to investigate the role of progesterone on uterine reactivity to exogenous oxytocin, concentration of oxytocin receptors, and gap junctions in the myometrium. Onapristone priming increased the ability of oxytocin to induce delivery during late pregnancy and before term by factors of ≥30 and approximately 10, respectively. The intrauterine pressure recording on day 43 after coitus revealed phasic, laborlike contractions in response to oxytocin in onapristone-treated animals, in contrast to tonic reactions in controls. The increase in the oxytocin response in onapristone-treated animals was not associated with an increase in myometrial oxytocin receptor concentrations either during late pregnancy or before term. By contrast, treatment with onapristone significantly decreased the input resistance of myometrial cells in guinea pigs in late pregnancy (43 ± 1 day after coitus) to the level of animals at term. This was associated with a marked increase in myometrial gap junctions stained with antibodies against connexin 43. These results indicate that progesterone may control myometrial reactivity to oxytocin in pregnant guinea pigs by effects on postreceptor events mainly by suppressing the gap junctions. (AM J OBSTET GYNECOL 1991;165:1760-70.)

Key words: Progesterone antagonists, oxytocin receptors, oxytocin sensitivity, gap junctions, pregnancy, guinea pig

The contractility of the uterus at the end of pregnancy depends on the release of endogenous uterotonic agents such as prostaglandins and oxytocin and an increase in responsiveness of the myometrium to these stimuli. Oxytocin is considered to play a major role in term labor. It is the most potent and specific natural uterotonic agent, and uterine contractions induced with oxytocin are identical to those occurring during spontaneous labor, providing the uterus is in a reactive state.1 The myometrium of various species, including humans, is most reactive to oxytocin either near or at the time of parturition.14 The enhanced myometrial responsiveness to oxytocin has been attributed to the increase in myometrial oxytocin receptor concentrations.2-4 Because there is no increase in oxytocin concentrations in the peripheral blood before the onset of labor in humans, it has been proposed that the increase in oxytocin receptor concentrations in myometrium

been shown in different species that in the course of pregnancy there is an increase in gap junctions, which are the structural basis of improved intercellular electrical coupling in the parturient myometrium. ⁵⁻⁷

The exact mechanism of the regulation of myometrial responsiveness during pregnancy is not clear and seems to be different in different species. In pregnant

and decidua at term is one of the primary factors lead-

ing to the initiation of parturition.⁸ However, it has also

trial responsiveness during pregnancy is not clear and seems to be different in different species. In pregnant rats the rapid increase in myometrial response to oxytocin that occurs about 1 day before term seems to be the result of a decrease in progesterone levels and an increase in estrogen levels in the peripheral blood.² By contrast, in humans^{1, 3} and guinea pigs^{8, 9} there is no decrease in serum progesterone levels before the onset of labor, and the myometrial responsiveness to oxytocin develops continuously in the course of pregnancy.

The novel 11β-aryl-substituted steroidal progesterone antagonist (antigestagen) mifepristone (RU 486)¹⁰ and the structurally related compound onapristone (ZK 98 299)¹¹ are specifically targeted to progesterone receptors and are noninvasive tools to investigate the role of progesterone in the onset of labor and myometrial response to oxytocic stimuli. A number in vitro¹² and in vivo studies in laboratory animals, ^{13,14} monkeys, ¹⁵ and humans¹⁶ have shown that antigestagens increase the myometrial responsiveness to prostaglandins and oxy-

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tocin. These studies indicate that the antigestagens might sensitize the uterus to uterotonic agents by increasing the receptor concentrations of oxytocin and prostaglandins and/or by postreceptor events including an elevation in gap junctions.

The current study was undertaken in late pregnancy and before term in guinea pigs to assess the oxytocin response, the oxytocin receptor numbers, and the gap junction formation after treatment with onapristone. In the preterm period of pregnancy the effect of onapristone on oxytocin response was compared with that of RU 486.

Material and methods

Animals. Female guinea pigs (Pirbright White, Charles River Wiga GmbH, Suthfeld, Germany) were kept in the presence of males and mated. The second day of vaginal opening in the fertile cycle was defined as day 1 after coitus. The animals were housed individually in an air-conditioned room (18° ± 1° C) with a light-dark regimen in which lights were on from 6 am to 6 pm. The weight of animals was between 800 and 1000 gm at 42 ± 2 days after coitus and between 1000 and 1400 gm at 60 ± 1 day after coitus. Spontaneous parturition in this strain of guinea pigs takes place overnight on day 67 or 68 ± 3 (median \pm SE).

Compounds and formulations. Onapristone (ZK 98 299) (11β-(4-dimethylaminophenyl)-17α-hydroxy-17β-(3-hydroxypropyl)-13α-methyl-4,9-gonadien-3-one) and mifepristone (RU 486) (11β-(4-dimethylaminophenyl)-17β-hydroxy-17-propinyl-4,9-estradien-3one) were dissolved in benzyl benzoate and then mixed with castor oil (1:2.25). Both antigestagens were synthesized at Schering AG (Berlin). Oxytocin (Syntocinon, Sandoz AG, Nürnberg, Germany) was diluted with 0.9% saline solution. Both the antigestagens and oxytocin were administered in 1.0 ml volume by subcutaneous injections. Control animals received the corresponding vehicle.

Induction of delivery in guinea pigs in late-pregnancy. Experiments were performed 43 ± 2 days after coitus. They were designed to establish the active threshold oxytocin dose that would induce delivery in the presence or absence (controls) of onapristone priming. The animals (n = 5 to 7 per group) were primed for 2 days (day 42 to 43 after coitus) with 10.0 mg onapristone per animal per day. Each oxytocin dose (dose range 300 to 30,000 mU per animal) was administered on the morning of day 45 after coitus either in serial injections (maximum of six injections) at 1-hour intervals (experiment 1) or, in a separate experiment (experiment 2), as a single subcutaneous injection. Controls were treated with onapristone vehicle and oxytocin. Previous studies have shown that the administration of 10.0 mg onapristone on days 42 and 43 after

coitus is effective in terminating pregnancy when given alone but the majority of animals do not deliver until the evening of day 46 after coitus^{13, 14} Animals that delivered before the first oxytocin injection were excluded from the experiment. The animals were observed continuously for 8 hours on the day of oxytocin treatment. On the following days cages were observed at least two times a day for delivered concept uses, placentas, and bleeding. The delivery of the first fetus was defined as the time of birth. Autopsy was performed on day 50 after coitus.

Induction of parturition studies. The aim of studies performed in the preterm pregnancy (61 \pm 1 day after coitus) was to evaluate the synergism of onapristone and RU 486 with oxytocin. Six to nine animals were used in each group (see Fig. 2). Onapristone (0.1 and 0.3 mg per animal) or RU 486 (0.3, 1.0, and 3.0 mg per animal) was given as a single injection on day 60 after coitus. Treatment with oxytocin was done on the morning of day 61 after coitus, 18 hours after antigestagen priming. Oxytocin was administered in serial subcutaneous injections of 25 mU per animal at 1-hour intervals until delivery or up to a total of six injections (maximum daily dose of 150 mU per animal). This dose of oxytocin was ineffective to induce preterm parturition without antigestagen priming. Previous experiments have shown that doses of >1000 mIU per animal per day (167 mU/hr) are necessary to induce birth during this period of pregnancy.9 The corresponding control groups were treated with the vehicles used for oxytocin or onapristone. The evaluation was performed as described. Autopsy was undertaken on day 70 after coitus.

Intrauterine pressure recording (day 42 after coitus). Three animals were used for each treatment and the control group. A sterile, rubber microballoon of about 0.5 ml volume filled with saline solution was inserted into the uterus on the morning of day 42 after coitus. The operation was performed with the animals under ether anesthesia. The balloon was attached to a polyethylene catheter connected to a Statham pressure transducer (Gould Inc., Oxnard, Calif.). The animals were treated on day 42 after coitus at 5 PM with onapristone 3.0 mg per animal (n = 1) and 10.0 mg per animal (n = 2) subcutaneously or with vehicle (controls). On the morning of day 43 after coitus, after an initial period of 30 minutes to record the spontaneous activity of the uterus, the animals received intravenous bolus injections of oxytocin in increasing doses of 3, 10, 30, 100, 300, 1000, and 3000 mU per animal at intervals ranging from 30 minutes to 1 hour, when the uterine activity had returned to its initial level. The intrauterine pressure was recorded over a period of 8 hours. The autopsy was performed on day 44 after coitus. The mean pressure (in millimeters of mercury) developed 1762 Chwalisz et al.

by the uterus per unit time was determined by measuring the area under the contraction curve for 10 minutes after each injection with a microcomputer-linked digitizing tablet.

Oxytocin receptor measurements. Myometrial oxytocin receptor concentrations were measured at various stages of pregnancy and post partum (for numbers, see Fig. 4) and after onapristone treatment during late pregnancy and before term. In late pregnancy the animals were treated on day 42 after coitus with subcutaneous onapristone, 10.0 mg per animal. Control animals were treated with vehicle. Each group consisted of four animals. The autopsy was performed on day 43 after coitus, 24 hours after treatment. In preterm pregnancy the guinea pigs were treated on day 59 after coitus at 5 PM either with 0.3 mg subcutaneous onapristone or with vehicle (controls). Eleven animals each were used for the treatment and vehicle control groups. The autopsy was undertaken 18 hours after treatment (day 60 after coitus).

Preparation of membrane fractions. During autopsy myometrium was separated from fat, decidual tissue, and fetal membranes. Myometrial specimens were immediately frozen in liquid nitrogen and stored at -80° C. Myometrial membrane fractions were prepared by modifications¹⁷ of the method described for rat myometrial membranes.¹⁸

Receptor binding assays. Myometrial membranes (30 to 100 µg protein) were incubated with 10 nmol/L tritium-oxytocin (specific activity 20 Ci/mmol, Amersham Buchler) at 30° C. The volume of the binding assay was 0.2 ml with the following components in the medium: 50 mmol/L Hepes and 10 mmol/L manganese chloride, pH 7.6. After 30-minute incubation, the medium was diluted with 5 ml of a cold filtration solution (0° C) containing 25 mmol/L Hepes, 2 mmol/L manganese chloride, and 0.1% bovine serum albumin. Membrane-bound tritiated hormone fraction was separated from the free hormone fraction by rapid filtration over cellulose acetate filters (0.22 µm). The filters were washed twice with the filtration medium and counted. Specific binding was obtained by the difference between tritium and ligand in the absence and presence of a 100-fold excess of unlabeled hormone. Equilibrium binding parameters were determined by a weighted nonlinear least-squares fit to logistic curves19 and by Scatchard analysis.

Electrophysiologic studies. The uterine samples from late-pregnant guinea pigs (n=8 per group) were used for the measurement of the input resistence of myometrial cells. Two groups were treated with subcutaneous onapristone 10.0 mg per animal on day 43 after coitus, and the measurements were performed 24

hours (group A) and 48 hours (group B) after the injection. The third group (group C) was treated for 2 days (day 43 to 44 after coitus) with subcutaneous onapristone 10.0 mg per animal per day. The autopsy was performed on day 45 after coitus. Two control groups were used. The first control group was treated with vehicle on day 43 and killed on day 44 after coitus. The second control group represents vehicle-treated prepartal animals on day 69 after coitus (approximately I to 2 days before the expected birth). The input resistance was measured from single smooth muscle cells with microelectrodes as used previously.¹²

Immunocytochemical staining. The animals (n = 8per group) were treated with subcutaneous onaprisotone 10.0 mg per animal per day on day 43 to 44 after coitus, and the autopsy was performed on day 45 after coitus. The control day group received vehicle. All tissues for immunocytochemical staining were rapidly frozen in liquid nitrogen and stored at -70° C until sectioning. The tissue was sectioned at a thickness of 7 to 8 µm. The polyclonal antibody that was used in this study was raised in rabbits and was directed against the cytoplasmic C terminal domain (amino acids 252 to 271) of connexin 43 (generously supplied by Elliot Hertzberg, Albert Einstein College of Medicine, New York). Sections of guinea pig heart were placed on each slide to validate the staining procedure because the guinea pig heart consistently stains with the connexin 43 antibody along the intercalated disks (see Fig. 8, C). After sections were rinsed in phosphate-buffered saline solution, 3% bovine serum albumin, and 1% Triton X-100, the primary antibody (1:250 dilution) against connexin 43 was placed on the tissue sections and they were incubated in a humidified chamber overnight at 4° C. The next day the slides were rinsed in phosphatebuffered saline solution (six times for 10 minutes each), and then a fluorescein isothiocyanate-labeled secondary antibody (goat antirabbit immunoglobulin G, Jackson Immunoresearch Laboratories, West Grove, Pa.) was added to the sections (1:200 dilution) and the preparation was incubated for 1 hour at room temperature. Afterward, the sections were again rinsed in fluorescein phosphate-buffered saline solution (six times for 10 minutes each), and then one or two drops of mounting medium (0.4% p-phenylenediamine in 0.1 mol/L sodium carbonate bufffer, pH 9, diluted 1:1 with 100% glycerol) was placed on each slide and a coverslip was applied. The sections were examined with a light microscope (Carl Zeiss, Inc., New York) ($\times 40$ to $\times 100$) with epifluorescence and filters for maximum fluorescein isothiocyanate fluorescence.

Statistical analysis. A two-sided t test ($\alpha = 0.05$) was used for statistical comparison of treatment groups

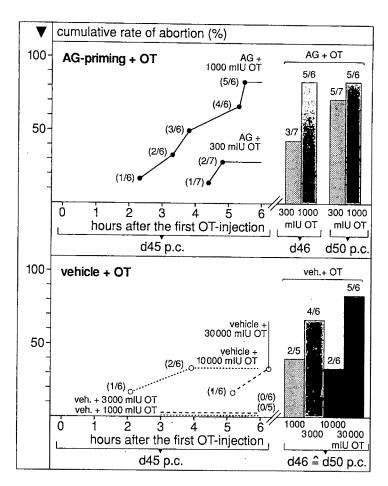


Fig. 1. Evaluation of active threshold oxytocin (OT) dose inducing abortion in presence (upper part) or absence (lower part) of onapristone (AG) priming in late-pregnant guinea pigs. Serial oxytocin injections. Animals (n = 6 to 7 per group) were treated as described in Material and methods. Results are expressed as cumulative rates of deliveries.

with the respective control groups to obtain oxytocin receptor measurements. The Wilcoxon rank sum test was used for the analysis of the electrophysiologic studies.

Results

Induction of delivery in late-pregnant guinea pigs

Experiment 1 (serial oxytocin injections). Oxytocin showed poor effectiveness in inducing delivery in the control groups. Only a few control animals delivered on day 45 after coitus after the highest oxytocin doses (10,000 and 30,000 mU) (Fig. 1). In the treatment groups delivery was induced on day 45 after coitus in five of six animals after serial oxytocin injections at 167 mU/hr (daily dose 1000 mU per animal) and in two of seven animals at 50 mU/hr (daily dose 300 mU per animal) (Fig. 1).

Experiment 2 (single oxytocin injection). No deliveries were observed in control animals on day 45 after coitus, and intact pregnancies were found in all animals during autopsy (day 50 after coitus). By contrast, four of five and two of five onapristone-primed animals gave birth on day 45 after coitus after 3000 and 1000 mU oxytocin, respectively. A single injection of 300 mU oxytocin was not effective in inducing delivery in onapristone-primed animals.

The results of both experiments indicate that in late pregnancy onapristone treatment resulted in lowering of the active threshold oxytocin dose by the factor of ≥30. However, this value should be considered a rough approximation because nonparallel dose-response curves were compared. The experimental protocol used in these studies, which included 2 days' antigestagen priming, was used in our laboratory for routine screening of various antigestagenic compounds. Other studies have demonstrated that 1-day priming with onapristone produced a similar increase in uterine responsiveness to uterotonic stimuli.20

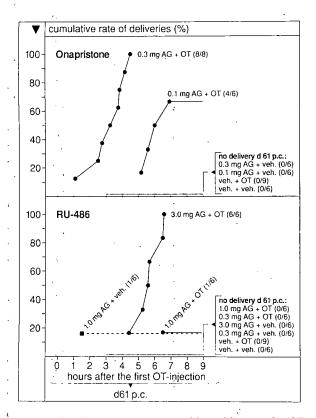


Fig. 2. Induction or preterm parturition with oxytocin (OT) after priming with antigestagens (AG) onapristone $(upper\ part)$ and RU 486 $(lower\ part)$. Oxytocin was given on day 61 after coitus in serial injections of 25.0 mU at 1-hour intervals (maximum of six injections) 18 hours after priming with onapristone, RU 486, or vehicle (controls) as described in Material and methods $(n=6\ to\ 9\ per\ group)$. Results are presented as cumulative rates of deliveries on day 61 after coitus.

Induction of parturition studies. Fig. 2 demonstrates a dose-dependent increase in oxytocin response for the induction of parturition after priming with onapristone and RU 486. Onapristone was approximately 10 times more effective than RU 486 in combination with oxytocin. After onapristone 0.3 mg per animal and RU 486 3.0 mg per animal, the normally ineffective dose of oxytocin (25 mU /hr) led to a delivery within a few hours of the start of oxytocin treatment in all animals. Significantly (p < 0.05, two-sided t test) shorter induction-delivery intervals and lower oxytocin consumption were seen in the group treated with 0.3 mg onapristone $(193 \pm 66 \text{ minutes and } 93.8 \pm 25.9 \text{ mU per animal},$ mean \pm SE) than in the group treated with 3.0 mg RU $486 (340 \pm 48.6 \text{ minutes and } 141.7 \pm 12.9 \text{ mU per}$ animal, mean \pm SE). Our previous studies have shown that at this stage of pregnancy oxytocin doses of >167 mU/hr (1000 mIU/day) were needed to achieve comparable effects in nontreated controls.9 This suggests an increase in uterine oxytocin responsiveness of approximately sevenfold to tenfold after antigestagen priming before term.

The doses of onapristone showing a synergism with oxytocin did not induce preterm birth (before day 64 after coitus) when given alone (control groups). Previous experiments have revealed that onapristone doses of ≥1.0 mg per animal are needed to induce preterm parturition without additional oxytocin treatment in guinea pigs. 18, 14 In control groups treated with RU 486 1:0 and 3.0 mg per animal approximately 50% of animals gave birth premature between the evening of day 61 or 62 and day 64 after coitus. In animals primed with onapristone or RU 486 no signs of labor were evident before the start of oxytocin treatment. However; antigestagen treatment produced bulging of fetal membranes through the cervix and vagina in some animals, which indicated that cervical softening and dilatation had occurred.

Intrauterine pressure recording. The responses to repeated oxytocin injections in the control and treated animals are presented in Fig. 3. In control animals little spontaneous uterine activity was observed before oxytocin treatment. After treatment with increasing doses of oxytocin there was an increase in uterine tone and sustained contractions of low amplitude. Even after the highest dose tested (3000 mU intravenous bolus), no regular, labor-type contractions of high amplitude were recorded. Furthermore, this dose did not induce abortion in control animals. Living fetuses were found during autopsy (day 44 after coitus) in the uteri of control animals.

In animals treated with onapristone irregular spontaneous contractions of low amplitude and relatively long duration were displayed after the injection of saline solution but before oxytocin treatment. Small doses of oxytocin (3 to 30 mU intravenous bolus) produced phasic contractions of high amplitude immediately after injection without affecting the uterine tone. The doses of 100 to 300 mU led to the rapid onset of highfrequency contractions of short duration and high amplitude that are similar to those observed during spontaneous labor. These doses led ultimately to abortion within 16 minutes to 2.5 hours in all animals treated. Analysis of the area under the contractility curves showed no basic difference between control and onapristone-treated animals. These data indicate that both groups are initially responsive to oxytocin, but the type of oxytocin-stimulated contractions differs (tonic contractions in controls vs phasic contractions in treated animals). Furthermore, the data show that oxytocin treatment leads to labor-type contractions and delivery only in the onapristone-treated animals.

Oxytocin receptor measurement

Oxytocin receptor concentrations during pregnancy and post partum (Fig. 4). The oxytocin receptor concentrations increased continuously (roughly tenfold) from day 30 to day 60 after coitus. The highest concentrations were

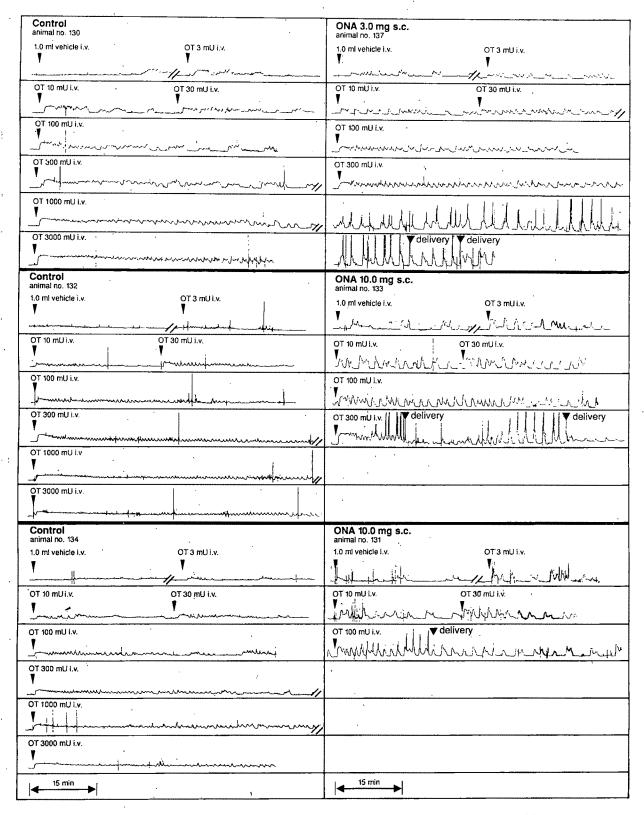


Fig. 3. Uterine contractility and oxytocin responses in pregnant guinea pigs pretreated with onapristone (ZK 98 299) (right panel) or vehicle (left panel). Animals were treated as described in Material and methods. Arrows indicate time of expulsion of fetuses.

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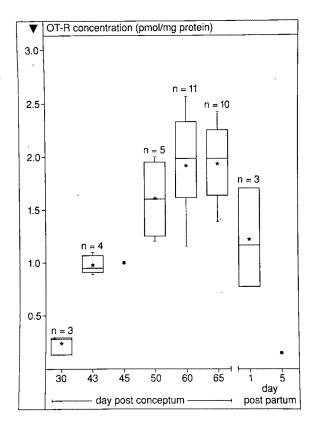


Fig. 4. Concentration of myometrial oxytocin receptors (OT-R) in guinea pig at various stages of pregnancy. Data are expressed as box plots. Vertical lines represent range from lowest to highest amount. Height of box, horizontal line, and asterisk describe interquartile range and median and mean values, respectively.

found in membranes derived from the myometrial samples of pregnant animals between day 50 and day 65 after coitus. Between day 60 and day 65 after coitus no significant change in oxytocin receptor concentration was found. Slightly lower oxytocin receptor concentrations were found on day 1 after birth.

Effect of onapristone treatment (Fig. 5). On day 60 after coitus there was no difference between the treatment and control groups (p > 0.05). However, on day 43 after coitus there was a significant (p < 0.05, two-sided t test) decrease in oxytocin receptor concentration after onapristone treatment. The equilibrium binding parameters from one onapristone-treated (0.3 mg per animal, day 59 after coitus) and one vehicle-treated animal were similar when analyzed by curve-fitting procedures and Scatchard analysis (Fig. 6). The following results were obtained in the treated and nontreated animals (values are mean \pm SE from three experiments): The apparent dissociation constants were 1.7 ± 0.1 and 2.6 ± 0.2 nmol/L, the apparent Hill coefficients were 1.2 ± 0.2 and 1.4 ± 0.2 , and the maximum binding

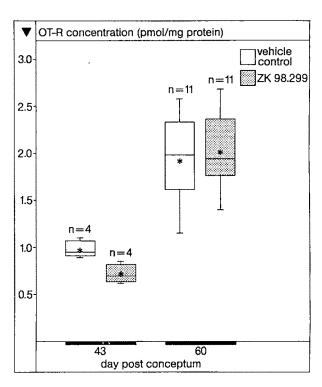


Fig. 5. Effect of treatment with onapristone (ZK 98 299) on myometrial oxytocin receptor (OT-R) concentrations in late-pregnant (day 43 after coitus) and preterm (day 60 after coitus) guinea pigs. Animals were treated as described in Material and methods. Data are presented as box plots as described for Fig. 4.

values were 2.9 ± 0.2 and 4.4 ± 0.1 pmol/mg protein, respectively.

Electrophysiologic studies. Input resistance values were significantly lower in control animals at term than in the late-pregnant controls. Onapristone treatment (groups A, B, and C) led to a significant (p < 0.05) decrease in input resistance, compared with that of the late-pregnant controls, to the level of control animals at term (Fig. 7). There was no difference between the treatment groups. When octanol (1.2 mmol/L), which is a specific gap junction uncoupling agent, was added to the recording bath, a dramatic increase in input resistance was measured in all treatment and control groups. This indicates that the decrease in input resistance that took place at term and after onapristone treatment was mainly due to the increase in gap junctions.

Immunocytochemistry. Fig. 8, A to C, shows the results of the myometrium and heart obtained with immunocytochemistry. Myometrial tissues from control guinea pigs at day 45 of gestation demonstrated little connexin 43 binding (Fig. 8, A). In contrast, myometria obtained from animals treated with onapristone for 48

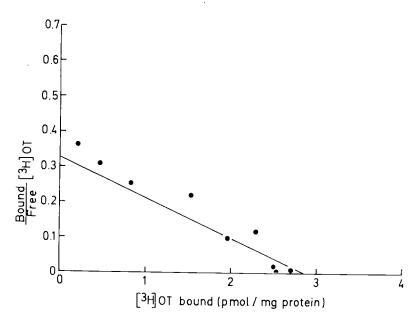


Fig. 6. Binding of ³H-oxytocin to myometrial membranes from guinea pigs treated with onapristone. Treatment and measurements were performed as described in Material and methods. Each point represents mean of three experimental values. Data are presented as Scatchard plot.

hours showed a profound increase in connexin 43 binding with punctate staining of rows of gap junctions, corresponding to the longitudinal orientation of the muscle cells (Fig. 8, B). For comparison, guinea pig heart is shown (Fig. 8, C). The connexin 43 staining between cardiac cells was limited to the region containing the intercalated discs, the position known to contain gap junctions.

Comment

The results of this study demonstrate that onapristone treatment substantially increased the ability of oxytocin to induce labor and delivery in pregnant guinea pigs. Comparable effects can be obtained before term (day 61 after coitus) with doses of this antigestagen that are approximately 30 times lower than those required in late pregnancy (43 ± 2 after coitus). This is remarkable in the light of minor differences in serum progesterone concentrations at both stages of pregnancy (approximately 1500 nmol/L 45 days after coitus vs approximately 1000 nmol/L 60 days after coitus²¹). The intrauterine pressure recording showed that treatment with onapristone converted the late pregnancy guinea pig uterus from an organ that responds tonically to oxytocin to one that reacts with phasic, laborlike contractions. Collectively, our data show that after antigestagen treatment the myometrium is more sensitive to oxytocin and is prepared for delivery.

A similar effect on uterine responsiveness to oxytocin has been shown in monkeys after RU 486 treatment. Sequential treatment with RU 486 and oxytocin was much more effective in inducing preterm parturition in monkeys than treatment with oxytocin or the antigestagen alone.15 An increase in responsiveness to oxytocin of the longitudinal and circular uterine muscle layers taken from pregnant rats treated with various antigestagenic compounds also has been demonstrated in vitro. 12 The results of our experiments are in contrast to a clinical study with RU 486 performed in the first trimester of pregnancy where the effect of antigestagen treatment on the uterine sensitivity to sulprostone (16phenoxy-tetranor-prostaglandin E2 methyl sulfonylamide) and oxytocin was investigated with intrauterine pressure recording.16 An increase in the uterine reactivity to sulprostone but not to oxytocin was found after RU 486 treatment in this study. Furthermore, no increase in the uterine responsiveness to oxytocin was found after treatment with the progesterone synthesis inhibitor epostane in early human pregnancy.22 However, the human uterus is not responsive to oxytocin during the first trimester of pregnancy, probably because oxytocin receptor concentrations are low at this time,3 whereas it is responsive to some degree to prostaglandins.1, 16 These observations would support the hypothesis that the antigestagens increase myometrial responsiveness at a postreceptor level, possibly by increasing gap junctions. Moreover, in early pregnancy antigestagens may act primarily on the endometrium and decidua rather than on the myometrium. 13, 14, 20

Previous studies have demonstrated that onapristone at doses used in this study precipitated cervical ripening

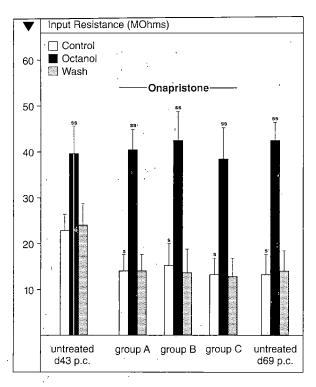


Fig. 7. Effect of onapristone treatment on input resistance of myometrial cells in late-pregnant guinea pigs. Animals were treated as described in Material and methods. White columns represent initial measurements. Black columns and dotted columns show input resistance values after in vitro treatment with octanol (1.2 mmol/L) and after tissues were washed with buffer, respectively. Values are mean \pm SE of cells that were impaled. S, Significantly different (p < 0.01) from nontreated control; ss, significant difference (p < 0.01) between control and exposure to octanol.

in both investigated periods of pregnancy. ^{13, 14, 23} Therefore the reduction of cervical resistance probably contributed in part to the effectiveness of oxytocin in inducing delivery.

The current study also demonstrates that the onapristone-induced increase in oxytocin response either during late pregnancy or before term was not due to the increase in oxytocin receptor numbers in the myometrium. On the contrary, onapristone treatment led to a significant reduction in oxytocin receptor concentration 43 days after coitus. Our data are in contrast to the results of a study performed in pregnant rabbits, which demonstrated an increase in myometrial oxytocin receptors after treatment with RU 486.²⁴ However, it is known that in this species, in contrast to guinea pigs, nonhuman primates, and humans, progesterone withdrawal leads to the onset of labor.^{1, 2} In our study oxytocin receptor measurements at different periods of pregnancy revealed a continuous, approximately

tenfold increase in receptor concentrations from early (30 days after coitus) to term (65 days after coitus) pregnancy in control guinea pigs. Similar changes in oxytocin receptors have been reported previously.4 In pregnant guinea pigs progesterone concentrations in peripheral blood show a moderate increase from early to term pregnancy and do not decrease before the onset of spontaneous labor.8, 21 The lack of an increase in oxytocin receptors after onapristone, together with the observation that rising progesterone levels throughout pregnancy accompanied the increase in oxytocin receptor concentrations, suggests that progesterone is not an inhibitor of myometrial oxytocin receptors in guinea pigs. Thus neither an acute increase in oxytocin receptors nor a change in oxytocin binding affinity is a prerequisite for the onset of labor in this species. This indicates that an increase in oxytocin sensitivity can occur preterm in this species without an increase in oxytocin receptors. The importance of changes in oxytocin receptors to the increase in oxytocin responsiveness has been questioned previously on the basis of the oxytocin sensitivity measurements in vitro of myometrial strips taken from pregnant rats at different stages of pregnancy.25 Our data provide further evidence that a rise in oxytocin receptor concentrations alone may not be the only mechanism for oxytocin to be effective. Therefore the question arises as to whether the increase in oxytocin receptor at term observed in this and other studies2-4 alone may explain the increse in myometrial sensitivity at term, which has been found in all species studied.

The electrophysiologic and immunocytochemical data convincingly demonstrate that there was a substantial increase in electrical coupling (reflected in decreased input resistance) and gap junctions in the myometrium after onapristone treatment in guinea pigs at late pregnancy. An enhanced electrical coupling as a result of the increase in density of gap junctions in the myometrium after treatment with various antigestagens has been demonstrated recently in rats.7, 12 Thus the increase in gap junction could be the major mechanism of increased responsiveness to oxytocin after antigestagen treatment. The observations that antigestagens increase the myometrial responsiveness not ony to oxytocin but also to prostaglandins and even to mechanical stimuli^{13, 14} support this conclusion. The results of intrauterine pressure measurements also are consistent with this view. Phasic and coordinated responses to oxytocin injections in onapristone-treated guinea pigs indicate that propagation of electrical events was improved by the treatment. This is the first study to describe the increase in gap junctions in the guinea pig after administration of antigestagens. Previous studies

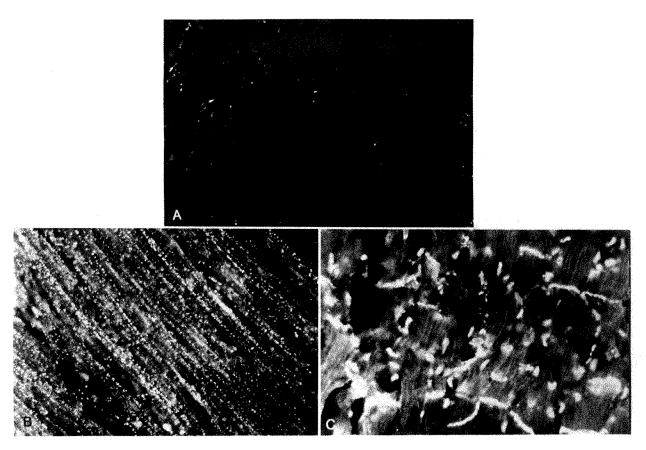


Fig. 8. Light micrograph of myometrial tissue from control animal on day 45 after coitus (A) and from animal after 2 days' treatment with 10.0 mg subcutaneous onapristone on day 43 to 44 after coitus (B) and of heart tissue from control pregnant guinea pig on day 45 after coitus (C) Treatments are explained in Material and methods. A, Note lack of staining as compared with B. B, Note abundance of bright fluorescent spots that represent gap junctions. C, Note immunostaining of intercalated discs, which confirm specificity of antibodies. (Original magnification × 1000.)

have described this phenomenon for rats after they received antigestagenic compounds.7.12 Furthermore, this is the first time connexin 43 binding has been demonstrated in the guinea pig myometrium although immunohistochemical studies of rat myometrium have been published.26, 17 However, none of the former studies quite demonstrate the dramatic differences shown in this study (Fig. 8, A vs B).

In animals primed with antigestagens no signs of labor were evident before oxytocin treatment. This observation is consistent with the concept that endogenous triggers of contractions such as prostaglandins or oxytocin are not under progesterone control in guinea pigs. 13, 14, 20 Taken together, our data provide further evidence that in guinea pigs progesterone may control uterine quiescence mainly by reducing the myometrial responsiveness, i.e., by the down-regulation of gap junctions but not by suppressing the release of endogenous uterine stimulants. This is contrary to the concept of Csapo,1 who postulated that progesterone controls both myometrial responsiveness and release of intrinsic uterotonic agents.

We are grateful to Dr. T. Louton for his expert help and advice with the statistical analysis. We thank Mrs. B. Kosub, Mrs. G. Bauer, Mr. S. Althof, and Mr. T. Goebel for their excellent technical assistance. We also acknowledge the assistance of Dr. N. Sakai with the electrophysiologic studies.

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Dose-related action of gonadotropin-releasing hormone on basal prostanoid production from the human term placenta

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The dose-related effect of gonadotropin-releasing hormone on placental prostanoids was studied with a perifusion system. Villous tissues were perifused with medium 199 (1 ml/hr) and at the beginning of the fifth hour, either 0, 10^{-10} , 10^{-8} , 10^{-8} , 10^{-7} , or 10^{-6} mol/L gonadotropin-releasing hormone was added to the medium of triplicate chambers. The concentration of prostaglandin E, prostaglandin F, 13,14-dihydro-15-keto-prostaglandin F_{2a}, 6-keto-prostaglandin F_{1a}, and thromboxane B₂ in the effluent medium, collected every hour, was determined by specific radioimmunoassay. The cumulative release after gonadotropin-releasing hormone treatment for each chamber was calculated, and replicate chambers were averaged. Linear regression analysis of the average for each dose from three different placentas was used to determine the dose-response relationship. Gonadotropin-releasing hormone significantly inhibited the release of placental prostaglandin E, prostaglandin F, and thromboxane B₂ in a dose-dependent fashion. Gonadotropin-releasing hormone had no significant effect on 13,14-dihydro-15-keto-prostaglandin F_{1a}, although there was an apparent increase in 13,14-dihydro-15-keto-prostaglandin F_{2a}. These data support the hypothesis that chorionic gonadotropin-releasing hormone inhibits prostanoid production from the placenta, which in turn may regulate various functions of prostanoids during pregnancy. (AM J OBSTET GYNECOL 1991;165:1771-6.)

Key words: Gonadotropin-releasing hormone, prostanoids, placenta, pregnancy, perifusion

Prostanoids in pregnancy are involved in hemodynamic regulation,^{1,2} ripening of the cervix,³ and initiation of labor.⁴ The placenta is a major source of prostanoid production during pregnancy.⁵⁻⁸ However, most studies related to prostanoid production have been carried out with amnion, chorion, or decidua, and little is known about placental production.

Previous studies of ours and others have demonstrated that synthetic gonadotropin-releasing hormone (GnRH) affects placental prostaglandins E (PGE) and F (PGF) and the 13,14-dihydro-15-keto metabolite of prostaglandin F (PGFM). Using a static explant culture system we found that either an inhibition or a stimulation of PGE, PGF, and PGFM release occurred, depending on the gestational age of the tissue and the dose of synthetic GnRH.^{5,9} Because GnRH can affect the maintenance of normal pregnancy, as demonstrated by the abortifacient actions of anti-GnRH antibody¹⁰ and GnRH antagonists or agonist in pregnant baboons, ¹¹⁻¹³ we hypothesized that GnRH may play

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a role in the regulation of prostanoid production in the term placenta. In addition, we found abnormally low levels of circulating immunoreactive GnRH during pregnancy to be associated with preterm labor and delivery.14 In patients who later had postterm pregnancies, we also found higher levels of immunoreactive GnRH.15 These studies strongly suggest that GnRHlike activity during pregnancy may play a role in the maintenance of pregnancy. Therefore we believed it important to investigate the precise action of GnRH on term placental prostanoid production. This study was undertaken with a dynamic perifusion system, and the dose-response relationship of GnRH on the release of placental prostaglandins (PGE, PGF, PGFM, 6-ketoprostaglandin F_{1a} [6-keto-PGF_{1a}] and thromboxane B₂ [TxB₂]) in the human term placenta was investigated.

Material and methods

Material. Medium 199 (×2) with Earle's modified salts, sodium bicarbonate (2.5 gm/L), and L-glutamine and without phenol red (Gibco, Long Island, N.Y.), Penicillin, streptomycin, crystalline bovine serum albumin, GnRH, and indomethacin (Sigma Chemical Co., St. Louis, Mo.) were used.

Placental perifusion. Human term placentas were obtained after vaginal delivery from patients having uncomplicated pregnancies with spontaneous onset of labor. Tissues were obtained in accordance with the institutional review board protocol. Small placental tis-

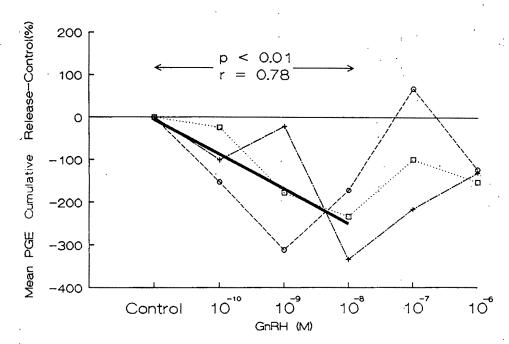


Fig. 1. Dose-related inhibitory action of GnRH on placental PGE production from term placenta (r = -0.78, p < 0.01). Each data point represents mean value of each placenta. *Thick solid line* is from linear regression analysis.

Table I. Percent prostanoid releases over fifth-hour release from perifused human term placenta

Hour	PG (mean ±		PG (mean ±		PGF (mean ±		6-Keto- (mean ±		Txi (mean ±	*
3	65.6	6.6	72.3	5.6	98.0	5.0	112.3	9.4	134.7	18.4
4	78.3	4.4	90.1	3.6	106.3	4.6	95.6	7.1	104.9	5.3
5	100.0	. 0.0	100.0	0.0	100.0	0.0	100.0	0.0	100,0	0.0
6	123.8	5.8	129.3	5.0	102.5	5.3	107.5	4.0	109.6	2.9
8	175.4	8.8	154.1	5.3	90.7	5.6	128.6	7.8	120.4	7.1
10	233.6	15.4	190.1	9.1	90.0	6.0	152.8	10.4	131.2	8.3
12	275.3	14.8	217.8	13.9	89.6	9.3	184.0	15.8	139.5	9.2

sue fragments (each about 25 mm³) were dissected, excluding decidua, chorionic plate, and large fetal vessels, and then rinsed with ice-cold normal saline solution until clear in color. Tissue fragments (total weight of approximately 1 gm per chamber) were placed in 20 tissue chambers in water bath at 37° C. Each chamber was perifused with medium 199 containing 0.05% bovine serum albumin, 100 U/ml penicillin, and 100 µg/ml streptomycin, hereafter collectively referred to as medium 199. Influx medium was aerated with 95% air and 5% carbon dioxide throughout the perifusion. The oxygen tension in the medium was maintained at 100 to 150 mm Hg, that is, similar to that of the placenta in vivo. Flow rate of the media was 1 ml/hr. After a 2hour equilibration period, samples were collected hourly for 12 hours into 12 × 75 mm glass tubes containing 0.1 ml indomethacin (1100 µg/ml). The dead volume of the system was approximately 1 ml; thus it took approximately 1 hour for the influx medium to pass through the perifusion tubing and the chamber and to be collected in the sample tube. The effluent of the 20 chambers was collected simultaneously with a fraction collector (Retriever III, ISCO, Lincoln, Neb.) adapted with 20-tube rack and manifold.

Effect of GnRH on placental prostanoid release. To study the effect of GnRH on placental prostanoid release, at the beginning of the fifth hour the medium in the experimental chambers was changed to medium 199 containing 10⁻¹⁰, 10⁻⁹, 10⁻⁸, 10⁻⁷, or 10⁻⁶ mol/L GnRH. Four replicate chambers were perifused with control medium; three replicate chambers were perifused for each of the experimental media. This experimental design was repeated with placental tissues from three different patients.

Prostanoid recovery and stability. Samples were stored at -20° C until assayed for PGE, PGF, PGFM,

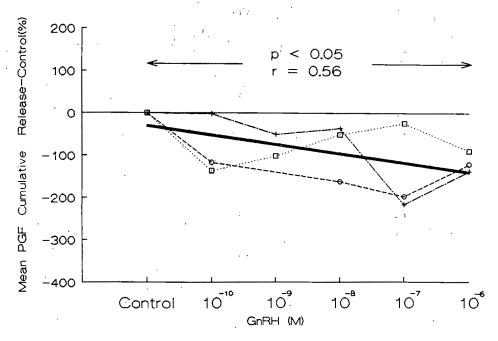


Fig. 2. Dose-related inhibitory action of GnRH on PGF production from term placenta (r = -0.56, p < 0.05). Each data point represents mean value of each placenta. Thick solid line is from linear regression analysis.

6-keto-PGF_{1α} and TxB₂. Recovery and stability of these prostanoids in this perifusion system and during storage were assessed by adding exogenous prostanoid to the influx medium and perifusing it through triplicate empty chambers and collecting samples as described above. In addition, samples were frozen and thawed repeatedly and reassayed. Prostanoid recovery and stability was 99%. Thus no data correction for procedural loss was necessary.

Radioimmunoassay. Radioimmunoassay was performed as described previously with specific exceptions as noted. After the initial pilot studies of prostanoid release of all hourly samples, samples for hours 3, 4, 5, 6, 8, 10, and 12 of perifusion were chosen for assay. All the samples from a given perifusion (placenta) were analyzed in the same assay.

PGE: A specific antiserum to PGE (Advanced Magnetics, Inc., Cambridge, Mass.) was used at a final dilution of 1/37,500. Label [5,6,8,11,12,14,15(n)-3H]-PGE₂, (Amersham Corp., Arlington Heights, Ill.), was added to every tube (25 pg). Assay sensitivity was 8 pg per tube, and the intraassay and interassay coefficients of variation were 7.8% and 9.8%, respectively.

PGF: A specific antiserum to PGF (Advanced Magnetics) was used at a final dilution of 1/60,000. Label $[5,6,8,9,11,12,14,15(n)-{}^{3}H]-PGF_{2\alpha}$ (Amersham) was added to every tube (25 pg). Assay sensitivity was 1.5 pg per tube, and the intraassay and interassay coefficients of variation were 11.0% and 10.4%, respectively.

PGFM: A specific antiserum to PGFM (Advanced Magnetics) was used at a final dilution of 1/4000. Label $[5,6,8,9,11,12,14(n)-{}^{3}H]-13,14-dihydro-15-keto-PGF_{2\alpha}$ (Amersham) was added to every tube (25 pg). Assay sensitivity was 7 pg per tube, and the intraassay and interassay coefficients of variation were 10.2% and 9.6%, respectively.

6-Keto-PGF_{1a}: A specific antiserum to 6-keto-PGF_{1a} (Advanced Magnetics) was used at a final dilution of 1/137,500. Label [5,6,8,9,11,12,14(n)- 3 H]-6-keto-PGF_{1 α} (Amersham) was added to every tube (25 pg). Assay sensitivity was 1 pg per tube and the intraassay and interassay coefficients of variation were 8.6% and 7.4%, respectively.

 TxB_2 : A specific antiserum to TxB_2 (Advanced Magnetics) was used at a final dilution of 1/70,000. Label [5,6,8,9,11,12,14(n)-3H]-TxB2 (Amersham) was added to every tube (25 pg). Assay sensitivity was 1.7 pg per tube, and the intraassay and interassay coefficients of variation were 5.3% and 5.7%, respectively.

Statistical analysis. Each prostanoid release for each chamber was first normalized to that of the fifth-hour release for that chamber. This functional normalization resulted in the least variation among replicate chambers. The integrated release from hours 6 through 12 was calculated for each chamber; mean values were obtained for each medium. The significance of doserelated effect of GnRH on prostanoid release was determined by performing linear regression analysis.

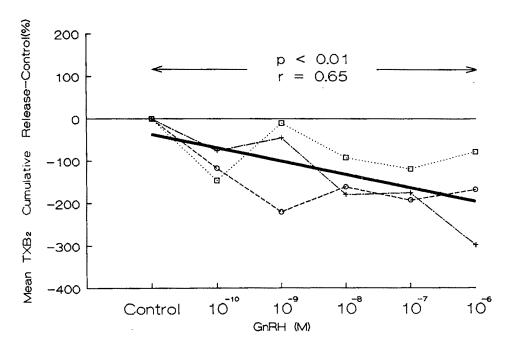


Fig. 3. Dose-related inhibitory action of GnRH on TxB_2 release from term placenta (r = -0.65, p < 0.01). Each data point represents mean value of each placenta. Thick solid line is from linear regression analysis.

Regression coefficients attaining a level of p < 0.05 were considered to be significant.

Results

The basal release of PGE, PGF, PGFM, TxB_2 , and 6-keto-PGF_{1 α} was 1165, 1212, 2625, 3249, and 237 pg/ml/gm tissue, respectively, at the fifth hour of perifusion. In control chambers the release of PGE, PGF, TxB_2 , and 6-keto-PGF_{1 α} increased over the 12 hours of perifusion (Table I). The addition of GnRH significantly inhibited PGE release from these human term placental explants in a dose-dependent fashion for concentrations up to 10^{-8} mol/L (r = -0.78, p < 0.01). However, at the higher concentration a lesser inhibition of the PGE release was noted, that is, there was a biphasic response having inhibition at the lower doses with relatively less effect at higher concentrations of GnRH ($>10^{-7}$ mol/L) returning toward control level (Fig. 1).

The release of PGF was also inhibited by GnRH, but no biphasic action of GnRH was noted in PGF release (Fig. 2). The inhibition of PGF by GnRH was dose related in the dose range studied, $(10^{-10} \text{ to } 10^{-6} \text{ mol/L}; r = -0.56, p < 0.05)$. Similarly to PGF, the release of TxB₂ from human term placental explants perifused with GnRH in medium 199 was inhibited in a dose-related fashion throughout the dose range studied (r = -0.65, p < 0.01) with increasing doses of GnRH (Fig. 3). In contrast, the release of PGFM did not decrease when placental explants were incubated with GnRH and actually increased (Fig. 4), yet this increase was not statistically significant. On the other

hand, 6-keto-PGF_{1 α} was unchanged by any dose of GnRH studied and had no noted changes throughout the perifusion period (Fig. 5).

Comment

These studies demonstrate that low doses of GnRH can inhibit placental PGE, PGF, and TxB2 production. In the case of PGE, the inhibitory effect of GnRH was observed over a shorter concentration range (10-10 to 10^{-8} mol/L) than that for PGF and TxB₂ (10^{-10} to 10^{-6} mol/L). It should be noted that the basal release of PGE, PGF, TxB₂, and 6-keto-PGF_{1a} increased over time in culture, whereas PGFM release was stable. It appears that the placental competence to produce PGFM is not stimulated by these culture conditions, yet for the other prostanoids it is increased. In addition, PGFM was the only prostanoid that increased after GnRH, although not significantly, whereas all the other prostanoids studied decreased. The stable release of PGFM and its differential response from that of PGF to enzyme inhibitors has been noted in our other studies (unpublished report, Kang et al.). These findings may indicate that GnRH acts to decrease placental PGF by inhibiting its production, enhancing its metabolism, or both.

In these studies we attempted to simulate a physiologic system by perifusing the medium with lower doses of GnRH in an air—carbon dioxide environment. The oxygen tension maintained was similar to that for the placenta in vivo. Even with a static culture system we and others have found that this environment enhances placental hormonal productions in vitro as compared with a higher oxygen tension. However, in our previous

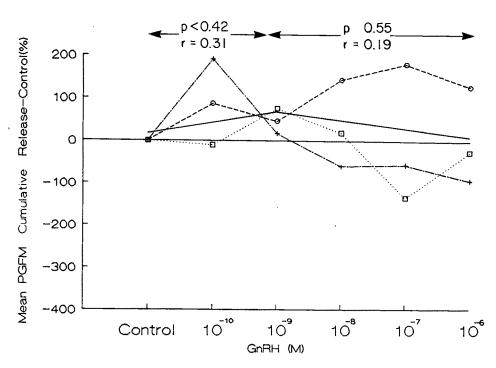


Fig. 4. Dose-related action of GnRH on PGFM release from term placenta. Each data point represents mean value of each placenta. Solid line is from linear regression analysis.

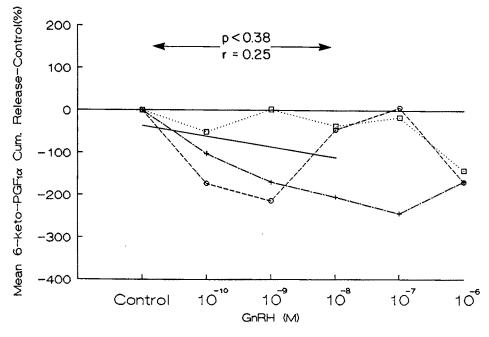


Fig. 5. Dose-related action of GnRH on 6-keto-PGF_{1α} release from term placenta. Each data point represents mean value of each placenta. Thick solid line is from linear regression analysis.

studies, which used a static culture system and GnRH at 10⁻⁶ mol/L, both inhibitory action and stimulatory action of GnRH on placental prostaglandin release were demonstrated.^{5,9} In the latter system products released from placental explants accumulated in the medium and thus the steroidal, growth factor, and other milieu was higher.

In this perifusion system the steroid and growth fac-

tor concentrations were very low because no exogenous hormones were added and the products of the tissue were continuously eluted. It is noteworthy that the prostanoid production in the perifusion system was lower than when basal steroid and growth factors were present. The magnitude of inhibition of prostanoids in these studies, although highly significant, was not marked, especially for PGF and TxB2, as has been previously observed for PGF in the first-trimester placenta with the static explant culture system. It may be that an even greater inhibitory or stimulatory action of GnRH on placental prostanoid productions may be observed in a higher estrogen or growth factor milieu. This possibility is being investigated.

These findings and our previous data demonstrate an inhibitory action of GnRH on prostanoid release in the lower, more physiologic concentrations of GnRH. On the other hand, these data appear to be in conflict with one other report in which no effect of 1.4 to 5.4×10^{-6} mol/L GnRH on PGF and a stimulatory effect of 100 nmol/L GnRH on PGF after a 4-hour incubation period were reported. However, it was difficult to compare these results because they were performed with a very different system, that is, 95% oxygen, different media, and different incubation time. It may be that in term placenta there is a biphasic response to GnRH modulated by steroids and growth factors.

In other recent studies of ours (unpublished data, Kang et al.) performed with this same perifusion system, we observed a decrease of PGF by addition of indomethacin, after which there was a decrease of PGFM. In our current study, it is interesting to note that GnRH decreased PGF release but was not accompanied by a decrease of PGFM. In contrast, an apparent increased release of PGFM was observed. Therefore this may actually reflect a greater physiologic response: PGFM was increased even when its substrate level was decreasing. Thus GnRH inhibition of PGF may reflect either or both decreased production and increased metabolism of PGF.

These data show that GnRH has a dose-related inhibitory effect on the placental PGE, PGF, and TxB2 release in vitro. This inhibitory action of GnRH may be a significant factor in the regulation of onset of labor, a theory supported by our findings that maternal circulating GnRH levels at 25 to 35 weeks' gestation were significantly higher in patients who later had post term pregnancies.15 In accordance with this finding, maternal circulating GnRH concentrations were significantly lower in four patients who later developed premature labor or delivery.14 Thus the inhibitory effect of GnRH on placental prostànoids may be a significant factor in the regulation of placental prostanoid production during pregnancy, allowing increased prostanoid release in later pregnancy when chorionic GnRH concentrations are lower.14.

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Circadian myometrial and endocrine rhythms in the pregnant rhesus macaque: Effects of constant light and timed melatonin infusion

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Six chronically catheterized rhesus macaques maintained on a 12-hour-light/dark cycle (lights on from 7 pm to 7 pm) showed a nocturnal uterine activity rhythm with peak contractile events between 9 and 11 pm (p < 0.05). In blood samples collected at 3-hour intervals over a 24-hour period, we determined that plasma melatonin and progesterone concentrations were elevated at night whereas estradiol, estrone, and cortisol reached peak concentrations in the early morning (p < 0.05). Lights were then left on for the remainder of the study. After 12 days in constant light, daily rhythms in uterine activity and plasma steriod levels were relatively unchanged, whereas melatonin concentrations were suppressed. Animals then received a timed infusion of melatonin (0.2 mg/kg/hr each day from 7 pm to 6 pm daily until delivery). The nocturnal uterine activity rhythm and the rhythms in plasma steroid concentrations were maintained. We conclude that the 24-hour patterns in maternal uterine activity and plasma steroid hormone levels are circadian rhythms generated by an endogenous biologic clock and do not appear to be driven by the pattern of melatonin in circulation. (Am J Obstet Gynecol. 1991;165:1777-84.)

Key words: Circadian rhythm, uterine activity, melatonin, rhesus macaque

Studies in the pregnant rhesus macaque demonstrate a 24-hour rhythm in uterine contractile activity in late gestation. 1-6 Peak contractile activity normally occurs during the night between 8 PM and 2 AM. Similar rhythms are presumed to occur in women,7-9 and further confirmation has been provided by a recent study by Germain et al. (Germain A. Personal communication). The mechanisms generating these rhythms and their potential role in the process of initiation of labor remain undefined. However, preliminary data in human and other nonhuman primates9-12 suggest that births are most common at night. Since uterine activity rhythms gradually increase in intensity and evolve into labor and delivery, it is conceivable that parturition may be an extension or amplification of these normally occurring rhythms. Thus 24-hour patterns of uterine activity could serve as a predictable model for the timing of the initiation of labor.

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Reprint requests: Charles A. Ducsay, PhD, Division of Perinatal Biology, School of Medicine, Loma Linda University, Loma Linda, CA 92350. 6/6/33637 Photoperiod appears to play a key role in entraining rhythms in the pregnant rhesus monkey during late gestation. We have previously demonstrated that both the uterine activity rhythm, and the timing of delivery were phase shifted by an 11-hour photoperiod shift. In addition, Figueroa et al. 13 have found that a 6-hour advance in lights off advanced the peak in myometrial contractility as indicated by changes in electromyographic activity. In addition to photoperiod entrainment of uterine activity, we have also shown that the light/dark cycle altered the 24-hour pattern of hormone concentrations in the maternal circulation such that within 1 week the various endocrine rhythms appeared to entrain to the new photoschedule. 14

In a variety of seasonal breeding species, the nocturnal melatonin rhythm mediates information about day length to time of onset and offset of reproductive function.15 During pregnancy, the melatonin rhythm conforms to the prevailing photoperiod in sheep¹⁶ and monkeys,6 with nocturnal plasma melatonin concentrations rising threefold to fivefold over daytime concentrations. At present, the specific target site for melatonin's action and the mechanism through which time cues affect normal physiologic function are unknown. We hypothesized that melatonin plays a key role in entraining the uterine endocrine system. The current study was designed to test the hypothesis that constant light with timed melatonin infusion will synchronize rhythms in uterine activity and endocrine secretion in the pregnant rhesus macaque during late gestation.

Material and methods

Animals and surgical procedures. Six rhesus macaques (Macaca mulatta) of known gestational age were obtained from the California Regional Primate Center, Davis, Calif. The animals were maintained in a controlled environment with a 12-hour-light/12-hourdark cycle during the initial portion of the study (lights on 7 AM to 7 PM; light intensity 350 lux). Animals were allowed free access to food while cleaning was performed in the morning on alternate days. Personnel were in the animal room at random times throughout the day and night for catheter maintenance, instrument calibration, and other routine tasks, as well as for the around-the-clock sampling protocols. A dim red light (<5 lux) remained on from 7 pm to 7 Am to facilitate the collection of blood samples at night. Between 115 and 120 days of gestation (term 167 days), maternal arterial, venous, and amniotic fluid catheters were surgically implanted as previously described. 4-6 After surgery, the animals were placed and maintained in a primate vest and tether system to which they were previously acclimated.¹⁷ All procedures were approved by the Institutional Animal Care and Use Committee.

Study protocol

Control photoperiod. Five to seven days after surgery, maternal arterial blood samples were collected at 3-hour intervals over a 24-hour period beginning at 9 AM, 2 hours after lights on. Additional samples were obtained ½ hour before and after lights on and lights off.

Constant light photoperiod. Immediately after the first sampling protocol, the room lights were left on for the remainder of the study. At 7 and 12 days after the start of constant light treatment, blood samples were again collected over a 24-hour period according to the sampling protocol described above.

Constant light with timed melatonin infusion. The animals remained in constant light and received a melatonin infusion (0.2 mg/kg/hr) each day from 7 pm to 6 AM until delivery. The infusion rate was modified from that of Foster et al. 18 After 7 days blood samples were again collected as described above. This sampling protocol was repeated 5 to 7 days later.

Uterine activity recording and analysis. Intraamniotic fluid pressure was monitored continuously throughout the study by connecting one of the saline solution—filled amniotic fluid catheters to an eight-channel polygraph (Gould, Cleveland) via a pressure transducer (Gould). The recorder interfaced with an IBM PC-AT microcomputer (Armonk, N.Y.) and a 12-bit analog-to-digital converter. The real-time data acquisition system and software used were described in detail elsewhere. For each uterine contraction, the amplitude, duration, time to peak amplitude, and integrated contraction area were recorded and stored on disk. Uterine activity data were analyzed as previously

described. 46 Briefly, the total contraction area for each hour of the day, defined as the hourly contraction area, was computed and used to quantify uterine activity and to evaluate the characteristics of the contractile rhythm for each animal. The mean hourly contraction area for each animal was defined as the sum of the hourly contraction areas divided by the total number of hours from the beginning of monitoring until delivery throughout the study. The hourly contraction area/mean hourly contraction area ratio was calculated for each hourly interval during a 24-hour cycle and the mean of the hourly contraction area/mean hourly contraction area ratio was used to normalize the data as previously described. 46

Assay procedures

Sample collection and storage. Maternal arterial blood samples (2 ml) were collected in chilled syringes containing ethylenediaminetetraacetic acid. Samples were centrifuged at 3500 rpm at 4° C. Plasma was separated and stored at -70° C until analyzed. Maternal erythrocytes were washed in saline solution, resuspended, and returned to the maternal circulation after each collection.

Hormone assays. Plasma concentrations of estrone, estradiol, and progesterone were assayed at the Oregon Regional Primate Research Center Radioimmunoassay Core Laboratory by previously described and validated radioimmunoassay methods. ²⁰⁻²² The intraassay and interassay coefficients of variation for the plasma estone, estradiol, and progesterone radioimmunoassays ranged from 7.8% to 12.7%.

Plasma dehydroepiandrosterone sulfate and cortisol levels were measured by direct radioimmunoassay as previously described and validated.²³ The intraassay and interassay coefficients of variation were <12%.

Adrenocorticotropic hormone was measured by direct radioimmunoassay with a commercial radioimmunoassay kit (IncStar Corp., Stillwater, Minn.). Measurement of adrenocorticotropic hormone in serial dilutions of pooled rhesus maternal plasma from late gestation gave a curve parallel to the adrenocorticotropic hormone standard curve. The intraassay and interassay coefficients of variation for rhesus maternal plasma were <10%. Assay sensitivity (defined as the smallest amount of adrenocorticotropic hormone per radioimmunoassay tube that reduces the number of counts per minute of labeled adrenocorticotropic hormone bound at zero mass by 2 SD) was 0.5 pg per tube.

Plasma melatonin was measured by radioimmunoassay with tritiated-melatonin (New England Nuclear, Boston) and a commercial antiserum (No. 704-6483, Guildhay Antisera, Guildford, Surrey, United Kingdom). Melatonin was extracted from plasma with 1 ml of glass-distilled chloroform. The chloroform phase was dried down (Hakke-Buchler vortex evaporator), rehydrated with 0.1 mol/L Tricine buffer (0.1 ml), and assayed as previously described.6 Recoveries were consistently >90% and therefore values were not corrected for procedural losses. Parallelism and quantitative recovery were confirmed in plasma pools collected from pregnant rhesus monkeys during the day and night. The limit of assay sensitivity (2 SD from buffer controls) was 0.6 pg per tube. The intraassay and interassay coefficients of variations were both <15%.

Statistical analysis. To detect 24-hour patterns, uterine activity and plasma hormone concentrations were compared with time of day by analysis of variance. Duncan's multiple range test was used for individual comparisons when the variance ratio (F) was statistically significant (p < 0.05).

Results

Uterine contractile rhythms. A rhythm in uterine contractile activity demonstrated a 24-hour periodicity (Fig. 1, upper panel); peak contractile activity occurred between 9 and 11 PM whereas the nadir occurred during the day (p < 0.05). The onset of elevated contractile activity appeared to coincide with the onset of darkness (represented by the dark bar). Daily rhythms in uterine contractile activity were not changed after exposure to constant light. Peak contractile events were observed between 9 PM and 1 AM whereas daytime activity was low (p < 0.05) (Fig. 1, middle panel). Daily melatonin infusions to the animals in constant light did not alter the uterine contractile rhythm when compared with that of previous control or constant light periods. Peak contractile activity again was observed between 9 PM and I AM with a nadir in activity in the daytime (p < 0.05). (Fig. 1, bottom panel).

The gestational age at delivery was 146.7 ± 3.5 days. After constant light with daily timed melatonin infusions, mean time of day of delivery was 7:53 PM ± 2 hours 52 minutes with a range from 3 PM to 2:30 AM.

Plasma melatonin rhythms. Plasma melatonin concentrations ranged from a nadir of 15 ± 2.4 at 7:30 PM to a peak of 57 ± 7 pg/ml at 9 PM during the control photoperiod (Fig. 2, upper panel). The nocturnal elevation in plasma melatonin level was sustained (at least from 9 PM to 12 midnight) during the dark phase whereas daytime levels were low (p < 0.01) In contrast, during constant light, melatonin concentrations throughout the period were not significantly different from daytime levels (Fig. 2, middle panel). Daily melatonin infusion (0.2 mg/kg/hr from 9 PM to 6 AM) reestablished a plasma melatonin rhythm (Fig. 2, bottom panel). Although daytime concentrations of plasma melatonin were similar to the values during the control photoperiod, the duration and amplitude of increased melatonin in circulation were greater than those ob-

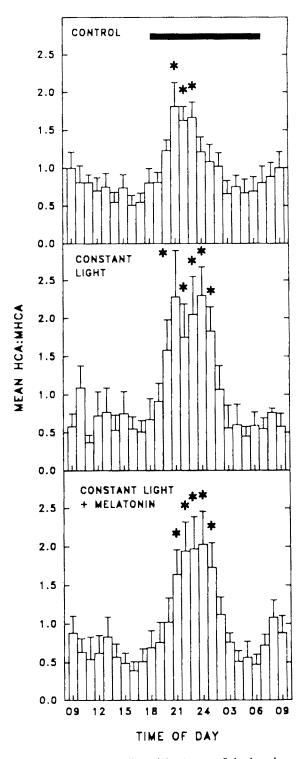


Fig. 1. Uterine contractile activity (mean of the hourly contraction area/mean hourly contraction area [HCA:MHCA] ratio) for chronically catheterized pregnant rhesus macaques. Alterations in uterine activity are represented with respect to time of day for 24 hours, during control photoperiod (upper panel), constant light (middle panel), and constant light with timed daily melatonin infusion (bottom panel). Hours refer to 24-hour clock. Periods of darkness (lights off from 7 PM to 7 AM) are indicated by solid bar in upper panel. Increases in uterine activity compared with low daytime levels are indicated by

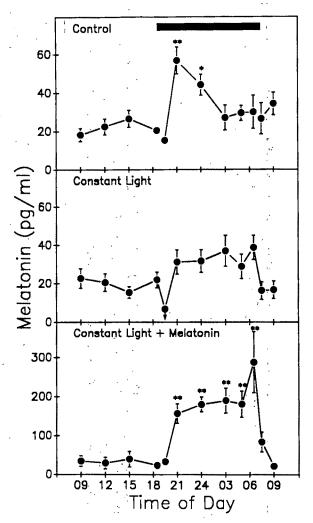


Fig. 2. Changes in maternal plasma melatonin concentrations are plotted with respect to time of day, during control photoperiod (upper panel), after 7 days of constant light (middle panel), and in constant light with timed daily melatonin infusion (bottom panel). Hours refer to 24-hour clock. Darkness during control period (lights off from 7 PM to 7 AM) is indicated by solid bar in upper panel. Values are mean \pm SE for six chronically catheterized pregnant rhesus macaques. Significant increases in concentrations compared with low daytime levels are indicated by asterisks (one asterisk, p < 0.05; two asterisks, p < 0.01). See Material and methods for details of blood sampling protocol and infusion procedure.

Plasma steroid rhythms. The 24-hour patterns of the various steroid hormones (mean ± SE) in the maternal circulation are illustrated in Figs. 3 to 7. Each figure presents data for the control photoperiod (upper panel), constant light (middle panel), and constant light with timed daily melatonin infusion (bottom panel). The solid bar in the upper panel of each figure indicates the period of darkness (lights off from 7 PM to 7 AM).

During the control photoperiod, plasma progesterone concentrations ranged from a nadir of 2.7 ± 0.3

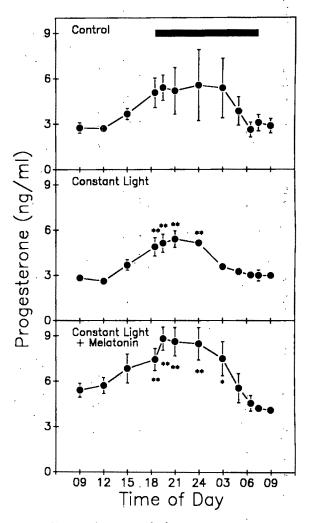


Fig. 3. Changes in maternal plasma progesterone concentrations throughout study. Refer to Fig. 2 legend for details.

ng/ml at 12 noon to a peak of 5.6 ± 2.3 ng/ml at 12 midnight (Fig. 3, upper panel). However, these differences did not reach statistical significance. In constant light (middle panel) progesterone levels ranged from a nadir of 2.6 ± 0.3 at 12 noon to a significant peak of 5.4 ± 0.5 ng/ml at 9 pm (p<0.01). Under constant light with melatonin treatment (bottom panel), the progesterone rhythm was maintained with a peak of 8.8 ± 0.8 ng/ml observed 7:30 pm (p<0.01).

Plasma estradiol concentrations during the control photoperiod range from 299 \pm 28 at 6:30 pm to 466 \pm 87 pg/ml at 5 AM (Fig. 4, upper panel). The rise in plasma during the early morning hours was not significant. In constant light, estradiol ranged from a nadir of 401 \pm 33 at 6:30 pm to a peak of 689 \pm 68 pg/ml at 7:30 AM (Fig. 4, middle panel). In addition to an elevation in mean estradiol levels, significant differences in estradiol concentrations between peak and nadir were observed (p < 0.01). With melatonin treat-

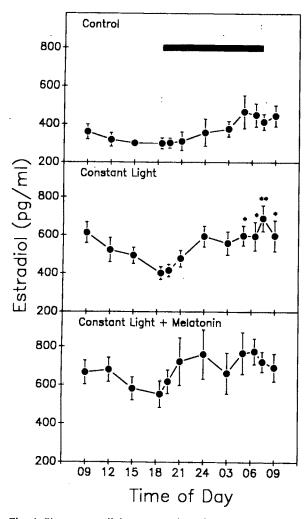


Fig. 4. Plasma estradiol concentrations throughout course of study. Fig. 2 legend contains details of study protocol.

ment, plasma estradiol ranged from 551 ± 69 at 6:30PM to 775 \pm 68 pg/ml at 6:30 AM (Fig. 4, bottom panel) but failed to demonstrate a significant rhythm as observed during the previous constant light period.

The plasma estradiol/progesterone ratio in circulation demonstrated a 24-hour rhythm throughout all phases of the study (Fig. 5). During the control photoperiod (upper panel) and constant light (middle panel) the nadir was observed at 7:30 PM, whereas the peak was noted between 6:30 and 7:30 ам. A similar pattern was observed during melatonin treatment (bottom panel). A significant difference between nadir and peak was observed throughout the study (p < 0.01).

During the control photoperiod, plasma estrone concentrations ranged from a nadir of 132 \pm 23 at 7:30 PM to a peak of 239 \pm 30 pg/ml at 9 AM (Fig. 6, upper panel). Concentrations were significantly elevated during the morning hours, 1/2 hour to 2 hours after lights on whereas the nadir occurred in the early evening (p < 0.05). During constant light this rhythm was main-

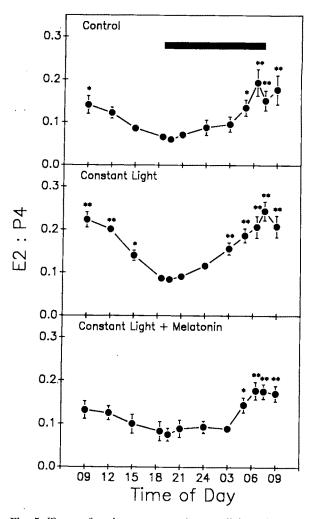


Fig. 5. Twenty-four-hour patterns in estradiol (E_2) /progesterone (P_4) ratio. See Fig. 2 legend for details.

tained and significant differences were noted between nadir (6:30 PM) and peak (6:30 to 7:30 AM) (p < 0.05). Although a similar pattern was observed with melatonin treatment the peak-to-nadir difference was not significant.

Alterations in plasma cortisol concentrations are illustrated in Fig. 7. Plasma corticol concentrations during the control photoperiod ranged from a nadir of 182 ± 12 at 6:30 pm to a peak of 319 ± 19 ng/ml at 6:30 AM. Peak concentrations in cortisol were significantly elevated in the early morning hours (p < 0.01, upper panel). Cortisol ranged from a nadir of 193 \pm 19 at 7:30 pm to a peak of $308 \pm 21 \text{ ng/ml}$ at 7:30 AM during constant light, and a circadian rhythm similar to that during the control phase was observed (middle panel). The mean values of cortisol in the early morning to noon were significantly increased as compared with the nadir value (p < 0.05). Cortisol ranged from a nadir of 192 \pm 7 at 7:30 pm to a peak of 295 \pm 23 ng/ml at 6:30 AM during constant light with melatonin supple-

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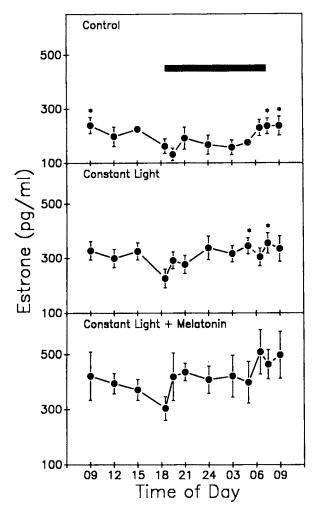


Fig. 6. Mean plasma concentrations of estrone during study. Refer to Fig. 2 legend for details.

mentation. The mean values of cortisol in the early morning were significantly elevated as compared with the nadir value (p < 0.05).

Maternal plasma adrenocorticotropic hormone concentrations ranged from 16 ± 2 to 38 ± 10 pg/ml throughout the study. Dehydroepiandrosterone sulfate levels ranged from 185 ± 35 to 240 ± 40 ng/ml. A 24-hour pattern was not observed in either of the hormones throughout the study (data not shown).

Comment

Photoperiod has a profound effect on the timing of birth in a variety of species, including humans. ⁶⁻¹² Since photoperiod also regulates the 24-hour rhythms in uterine activity in pregnant rhesus macaques, ^{6-13, 14} we tested the hypothesis that the uterine activity rhythm is endogenous, that is, it persists under constant environmental conditions. To our knowledge these studies are the first to examine the effect of continuous light on endocrine rhythms associated with pregnancy,

plasma melatonin patterns, and uterine activity. We found that the rhythms in uterine contractile activity and plasma steroid hormone concentrations remained relatively unchanged after exposure to constant light, suggesting that these rhythms are driven by a circadian mechanism involving an endogenous biologic clock. Further, the uterine activity rhythm remained unchanged even though constant light abolished the melatonin rhythm, suggesting that melatonin does not drive the uterine activity pattern. This concept is further supported by the lack of effect of a daily melatonin infusion on uterine activity and plasma hormone concentrations.

Uterine activity data from our current study are in agreement with those previously demonstrated by us⁴⁻⁶ and other investigations.^{2, 3} Contractile activity in the rhesus macaque follows a well-established pattern, with peak activity during the late night or early morning hours. The photoperiod used in these studies is similar to that of Taylor et al.² In earlier studies Harbert and Spisso¹ used 12-hour-light/12-hour-dark cycles as well. However, in their study the lights were on from 4 AM to 4 PM and peak contractile activity occurred approximately during daylight hours. Differences in the time of peak contractile activity among these studies appear to be the result of an entraining effect of the light/dark cycle.^{4, 6, 13}

There is abundant evidence in human and nonhuman primates that light/dark cycles are the principal zeitgebers, capable of entraining physiologic circadian rhythms.24 In seasonal breeding species, including the rhesus macaques, photoperiod control of the circadian pineal melatonin rhythm is suggested to regulate reproductive function and endocrine secretion.25 In our previous studies in the pregnant rhesus macaque, 6, 14 when the light cycle was shifted, a parallel shift in melatonin rhythms was observed along with alterations in other maternal hormones and uterine activity. These findings raise the possibility that in the rhesus, as well as other seasonal breeders,15 the 24-hour melatonin rhythm conveys information about the photoperiod and synchronizes various maternal endocrine rhythms to the light/dark cycle. In the current study plasma melatonin levels in the maternal circulation demonstrated a 24-hour rhythm during the control photoperiod with peak concentrations attained at 9 PM whereas daytime concentrations were low. However, the absence of a melatonin rhythm under constant light conditions (mean values over 24 hours were similar to daytime levels) was associated with the persistence of a rhythm in uterine contractile activity. During the melatonin infusion, mean levels exceeded those observed during the peak of the nighttime rise during the control photoperiod by approximately fourfold. Studies by Ebling et al.26 in the sheep and Goldman et al.27 in the hamster have shown that the amplitude of the nocturnal rise in melatonin is not what determines the photoperiodic response. The duration of the rise must be sufficient to elicit a response. The duration of the melatonin rise induced by the infusion was sufficient when compared with that of the controls. These data fail to support our original hypothesis and indicate that melatonin may not play a key role in entraining the uterine endocrine system under constant light conditions. Moreover, the findings raise the question of whether melatonin plays a role in the photoperiod-mediated process that times the onset of labor.

A number of hypotheses have been proposed to explain the factors responsible for the generation of 24hour patterns in plasma steroid concentrations in the pregnant rhesus macaque. Sholl et al.28 suggested that the nocturnal increase in maternal progesterone levels reflects an alteration in placental secretion, as a result of either changes in blood flow or altered placental steroidogenesis. The blood flow hypothesis seems unlikely because uterine contractions occurring maximally at night2-6 decrease placental blood flow and would limit the availability of substrate for placental steroidogenesis at a time when progesterone levels are rising. A more plausible hypothesis was proposed by Hess et al.29 They postulated that the diurnal cortisol secretion by the maternal adrenal changes the displacement of progesterone from common binding sites on plasma proteins. Therefore the inverse patterns in plasma progesterone and cortisol are the result of altered binding of progesterone to plasma proteins because adrenal activity (cortisol secretion) varied throughout the day. However, the factors responsible for the daily alterations in adrenal activity remain undefined.

The circadian uterine activity rhythm may depend on rhythms in plasma steroid levels. Estrogen concentrations have been demonstrated to have profound effects on uterine contractility. Elevated estradiol/progesterone ratios have been associated with increased contractile activity in the rabbit30 and sheep.31 On the basis of these studies, data in the rhesus monkey appear on the surface to contradict findings in other species. As shown in Fig. 5, the peak estradiol/progesterone ratio was observed in the early morning followed by a nadir in the evening. The nighttime peak in the uterine activity rhythm (between 8 PM and 1 AM) occurs when estradiol/progesterone levels are around nadir and progesterone levels are elevated, that is, the estradiol/progesterone ratio is at its lowest (Fig. 5). However, Roberts et al.32 demonstrated that estrogen increases myometrial adrenergic receptors in as little as 6 hours and with maximum responsiveness at 12 hours. Thus peak levels in estrogen may require up to 12 hours to maximally influence myometrial responsiveness to circulating uterotonins. This is precisely the time

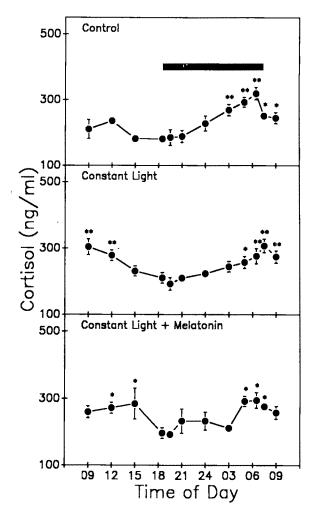


Fig. 7. Changes in plasma cortisol concentrations throughout study. See legend to Fig. 2 for details.

lag between the peak in estrogen concentrations in maternal plasma and the first significant increases in uterine contractile activity. Thus the circadian rhythm in estrogen levels or the estrogen/progesterone ratio may still play a crucial role in the regulation of myometrial contractile rhythms.

Data from the current study confirm that 24-hour rhythms in circulating plasma steroid levels are a characteristic of pregnancy in the rhesus macaque during late gestation^{6, 14, 33, 34} and extend these observations to include maintenance of these rhythms under constant conditions. Taken together with our previous observation that these rhythms reentrain to an altered photoperiod, these results suggest that photoperiod may entrain the rhythms, but the rhythms are ultimately the result of an endogenous pacemaker.

In conclusion, photoperiod has been shown to profoundly affect endocrine rhythms^{6, 14} and uterine contractile rhythms.6, 13 In this study uterine activity and plasma steroid hormone rhythms were found to persist under constant conditions. These data further strengthen the concept that these 24-hour patterns are circadian rhythms generated by an endogenous biologic clock.

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Effects of intravenous cocaine on reproductive function in the mated rabbit

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A dramatic increase in the use of crack cocaine by young women has resulted in the exposure of a substantial number of fetuses to the drug in utero. The goal of these experiments was to investigate the effects of intravenous cocaine (a model for crack) on reproductive function in the female rabbit. We used two protocols: (1) single daily injections over the course of pregnancy ("single daily" protocol), with animals examined on day 1, 8, 15, 22, or 29, and (2) six hourly injections on the day of mating ("binge" protocol). The maximum tolerated dose of cocaine, 4 mg/kg, was administered at 0.5 ml/min. The single daily protocol increased ovulation but had no effect on fetal and placental weights or preterm delivery. The binge protocol significantly reduced in vitro development of retrieved embryos but did not affect implantation assessed on day 8 of pregnancy. (AM J OBSTET GYNECOL 1991;165:1785-90.)

Key words: Cocaine, rabbit, ovulation, implantation, pregnancy

Crack, the smoked form of cocaine, is used by a substantial percent of pregnant women,1 and use of the drug is associated with decreased birth weight and increased preterm delivery.2.3 The pharmacokinetics of cocaine is dependent on the route of administration. Both smoking crack and intravenously injecting cocaine produce extremely high circulating levels of the drug almost instantaneously, and nasal insufflation ("snorting") and subcutaneous administration yield lower circulating levels that reach a peak 30 minutes to 1 hour after administration.4 Most animal studies of the effects of cocaine on female reproduction have used the subcutaneous route,5,6 which is a good model for nasal insufflation but may not produce the effects of the high levels and rapid increase in plasma cocaine that are seen with crack use.

The overall goal of these experiments was to investigate the effects of intravenous cocaine (a model for crack) on reproductive function in the female rabbit. We have modeled two patterns of crack use, daily usage and less frequent binging, with two experimental protocols: (1) single daily injections over the course of pregnancy (single daily) and (2) six hourly injections on the day of mating (binge). Control rabbits were in-

jected with equal volumes of the saline solution vehicle. The specific purposes of the single daily protocol were to examine ovulation, fetal, placental, and corpora lutea weights, and progesterone levels in animals treated with either cocaine or saline solution. We also examined the effects of the higher-dose binge protocol on early reproductive events, such as ovulation, early embryonic development, and implantation.

Material and methods

Animals. Adult New Zealand White does weighing 3.5 to 6 kg were obtained from Bunnyville Farms (Littlestown, Pa.) and were housed individually in our animal quarters for 3 weeks before use under controlled light (14 hours light-10 hours dark; lights on at 7 AM) and temperature (21.1° to 22.2° C). Animals had free access to rabbit chow and water. Studies were performed in accordance with the Guidelines for the Use of Experimental Animals at the Johns Hopkins University. Each female rabbit was brought to a male's cage, and the pair was observed for successful mating. If no attempt at mating occurred within 1 minute, the female was placed in the cage of another male. Mating was confirmed by observation of sperm in a vaginal smear. The day of mating was designated day 0 of pregnancy (term is normally day:31 or 32). Four males of established fertility were used in the experiments. The female rabbit was weighed immediately after mating, and cocaine (Sigma Chemical Co., St. Louis) or saline solution vehicle was injected into the marginal ear vein. Cocaine solutions were made fresh weekly in sterile saline solution and were stored in the dark. The delivered dose of cocaine was 4.0 mg/kg, which was administered by infusion pump in a volume of 0.25 ml/kg at a rate of 0.5 ml/min. Approximately 10% of the rabbits expe-

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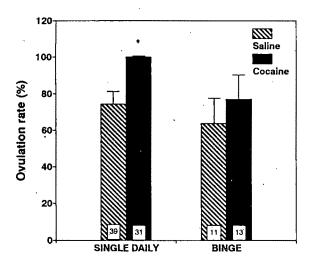


Fig. 1. Ovulation rates in mated rabbits subjected to single daily and binge protocols. Ovulation rate = (Number of animals ovulating/Number of animals mated) \times 100. All animals that received cocaine in single daily protocol ovulated (asterisk, p < 0.01, compared with saline solution controls). There were no differences between saline solution and cocaine groups with binge protocol. Number of animals mated is indicated within bar.

rienced drug-induced convulsions that resulted in traumatic vertebral fracture and lower limb paralysis. Those animals were killed with pentobarbital sodium.

Single daily protocol. Rabbits were weighed, and the cocaine (n = 31 rabbits) or saline solution (n = 39) was injected daily between 8 and 11 AM. On day 1, 8, 15, 22, or 29 of pregnancy, 24 hours after the last cocaine or saline injection, rabbits (n = 5 to 8 per treatment for each day examined) were anesthetized with pentobarbital sodium (32 mg/kg), and a laparotomy was performed. Heparinized blood samples were obtained from each cannulated ovarian vein8 and from the periphery (vena cava) for progesterone determination. Plasma samples were stored at -70° C until they were assayed. The numbers of corpora lutea and unruptured follicles ≥1.5 mm in diameter were recorded on day 1. On subsequent days ovaries and pregnant uteri were removed; corpora lutea were dissected from each ovary, pooled, and weighed; and the number of implantations per side was ascertained. On days 15, 22, and 29 fetuses and placentas were separated and those contained in each uterine horn were pooled and weighed. Ten other females were mated and weighed daily but were not restrained or injected. These untreated controls were killed on day 29, and the corpora lutea, fetuses, and placentas were counted and weighed as previously described.

Binge protocol. Animals were mated and weighed, and a blood sample was drawn from the marginal vein of one ear (cocaine n = 9; saline solution n = 6 rabbits). The first dose of cocaine or saline solution was

administered as previously described through the marginal ear vein of the contralateral ear. Blood sampling and cocaine or saline injection were repeated once an hour for a total of six injections. Plasma was stored at -70° C until it was assayed.

Twenty-four hours after mating (day 1), a peripheral blood sample was drawn, animals were anesthetized with pentobarbital sodium, and a laparotomy was performed. A blood sample was collected from each ovarian vein, and the ovary, fallopian tube, and proximal portion of the uterine horn were removed en bloc from each side and placed in saline solution. Ovaries were inspected for the number of corpora lutea and unruptured large follicles (diameter ≥ 1.5 mm). Ova and embryos were recovered by flushing fallopian tubes with Ham's F-10 medium (Gibco, Grand Island, N.Y.). Embryos recovered from a single tube were cultured together in vitro in Falcon organ culture dishes (Becton Dickinson, Lincoln Park, N.J.) containing 0.7 ml Ham's F-10 medium plus 20% fetal calf serum (Gibco), 0.1 mg/ml gentamicin (Gibco), and 31 µg/ml penicillin-G (Sigma). Embryonic development was assessed daily for six days in accordance with a treatment-blind protocol.

To assess the effects of the binge treatment on implantation, the binge protocol was repeated with another group of rabbits (cocaine n=4; saline solution n=4). These animals were not disturbed after mating and repeated dose administration. On day 8 of pregnancy a peripheral blood sample was taken from the lateral ear vein, and the implantations per uterine horn were counted. Corpora lutea were also counted, dissected from each ovary, pooled, and weighed.

Progesterone determinations. Progesterone in peripheral and ovarian vein plasma samples were determined in duplicate by a solid-phase radioimmunoassay (Diagnostic Products, Los Angeles).8

Data analysis. The unit of analysis for most measurements was the side: Rabbits have two cervices, and the left and right reproductive units (ovary, fallopian tube, and uterine horn) are totally separate. Corpora lutea and fetal and placental weights and numbers, as well as ovarian vein progesterone determinations, were treated as repeated measures for left and right sides within each rabbit. These data were analyzed by twofactor analysis of variance with one repeated measure (left and right sides). Ovulation rate (the percent of mated rabbits that ovulated), ovulatory efficiency (the percent of follicles ≥ 1.5 mm in diameter that ovulated by 24 hours after mating), preimplantation development, and implantation index (the number of implantations per number of corpora lutea) were analyzed by use of 2 × C contingency tables. Yates' correction was used for 2 × 2 tables. Rabbit weight gain was assessed by multivariate regression; the day of pregnancy, initial weight, and treatment group (cocaine, saline solution,

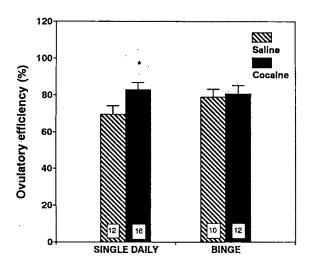


Fig. 2. Ovulatory efficiency among animals ovulating, as assessed on day 1 of pregnancy. Ovulatory efficiency = (Number of corpora lutea/[Number of corpora lutea + follicles ≥ 1.5 mm]) × 100. Ovulatory efficiency in animals receiving single injection of cocaine was significantly higher (asterisk, p < 0.05) than that of saline solution controls. There was no difference in ovulatory efficiency with the binge protocol between animals treated with cocaine and those that received saline solution. Number of ovaries examined is indicated within bars.

or untreated control) were used as the independent variables. Differences with p < 0.05 were considered significant.

Results

Single daily protocol

Ovulation. All the animals receiving daily cocaine injections ovulated, as compared with only 74% of the saline solution controls (p < 0.01, Fig. 1). Moreover, ovulatory efficiency in animals that ovulated was significantly higher in animals receiving cocaine than in saline solution controls (cocaine, 101 ovulations from 122 large follicles; saline solution, 68/98; p < 0.05, Fig. 2). Cocaine administered in the single daily protocol did not stimulate ovulation in unmated animals (data not shown).

Weight gain. Data from groups of rabbits subjected to laparotomy on day 22 and day 29 of pregnancy were combined, and the analysis is based on data through day 22. No change in the pattern of weight gain was seen between day 22 and day 29. Weight gain was reduced in the cocaine-treated animals when compared with the saline solution-treated controls (p < 0.0001, Fig. 3). However, the untreated animals gained weight at a significantly higher rate than did either of the treated groups (p < 0.0001).

Fetal and placental weights. In spite of the difference in maternal weight gain, average fetal and placental weights on days 15 and 22 of pregnancy were similar

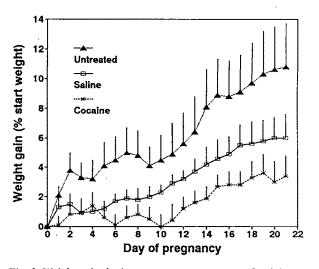


Fig. 3. Weight gain during pregnancy as percent of weight on day of mating. Animals were weighed daily: untreated (n = 5), single daily injection with saline (n = 8), single daily injection with cocaine (n = 9). Restraint and injection decreased weight gain as compared with that of untreated animals (p < 0.0001), and cocaine-treated animals gained less weight than did saline solution-treated controls (p < 0.0001).

in animals injected daily with saline solution or cocaine (Table I). On day 29 fetal and placental weights were similar in both untreated animals (fetal 37.8 ± 1.5 , placental 4.28 ± 0.19) and animals injected with cocaine or saline (Table I). The average number of fetuses per uterine horn was not affected by cocaine treatment (data not shown). Delivery in this strain of rabbits is expected on day 31 or 32 of pregnancy9; however, none of the rabbits subjected to the single daily protocol delivered by day 29.

Corpora lutea weight and progesterone levels. No significant differences in the average weight (Table II) or number of corpora lutea per ovary (data not shown) were found between cocaine- and saline solutiontreated groups. Average corpora lutea weight in the day 29 untreated control group was 21.2 ± 0.73 , which was similar to weights for both the treated groups.

Peripheral and ovarian venous progesterone levels in treated animals were examined on days 1, 8, 15, 22, and 29 of pregnancy (Table II). Peripheral progesterone levels reached a peak at midgestation and then declined. In contrast, ovarian vein progesterone levels rose dramatically from day 1 to day 8 and remained high through day 29. There were no significant differences between the cocaine- and saline-treated groups with respect to peripheral or ovarian vein progesterone levels.

Binge protocol. There were no differences in occurrence of ovulation (Fig. 1) or in ovulatory efficiency between cocaine (62/77 follicles) and saline solution (63/80 follicles) groups (Fig. 2). Fifty embryos were

Table I. Average fetal and placental weights with day of pregnancy in rabbits treated with saline solution or cocaine in single daily protocol*

	1	Day 15		Day	22	Day	29,
	Saline solution	· .	Cocaine	Saline solution	Cocaine	Saline solution	Cocaine
Fetal weight (gm)			: '				ţ-
Average	0.38		0.38	7.78	7.46	. 42.9	39.9
SEM	0.012		0.018	0.26	0.12	1.82	2.7
n .	10	. ~	8 .	8	8	8	9
Placental weight (gm)					,		
Average	0.72		0.86	3.39	3.04	5.4	4.84
SEM SEM	0.045		0.043	0.24	0.11	0.31	0.36
n ,	. 10		8	8	8	8	9

^{*}Averages are per side and n is number of sides.

Table II. Average corpora lutea weights and peripheral and ovarian vein progesterone levels with day of pregnancy in rabbits treated with saline solution or cocaine in single daily protocol*

	Day 1		Dáy 8 .		Day 15		Day 22		Day 29	
	Saline solution	. Cocaine	Saline solution	Cocaine	Saline solution	Cocaine	Saline solution	Cocaine	Saline solution	Cocaine
Corpora lutea weight		•	, ",				· ·			٠.
(mg)								•		: .
Average	1.6	1.53	. 13.2	13.8	20.7	21.1	18.4	19.5	23.6	20.1
SEM	0.15	0.20	0.44	0.44	0.71	0.86	0.66	1.13	0.86	0.64
\hat{n}	10	10	12 .	10	8	8	8	8	. 8	` 8
Peripheral progesterone										
(ng/ml)		'	•							
Average	1.71	1.66	12.9	10.12	20.4	18.9	11.63	7.3	7.18	10.0
SEM	0.24	0.35	3.35	1.53	5.36	1.92	2.81	1.2	3.06	2.91
\boldsymbol{n}	7	7	8 -	7	5	3	3	. 4	4	. 4
Ovarian vein progester- one (ng/ml)										ş '
Average	59.50	70.09	1074	1110	1239	1212	.1142	1348	1104	1002
SEM	10.54	12.83	118	159	163	157	249	191	174	183
n	13	. 14	15	13	103	8	. 8	8	8	8
Vein progesterone per	10	,		10		Ü	Ü	Ü	Ū	
weight of corpora lu-	•				:					
tea (ng/ml/mg)	*		·				4			-
Average	8.00	11.15	15.11	18.54	7.89	11.50	8.52	12.98	9.53	11.99
SEM	2.12	2.77	1.13	3.01	. 0.84	1.74	1.36	2.20	1.08	2.27
n · · ·	10	10	12	10	8	8	8	8	8	7

^{*}Averages are per side and n is number of sides, except for peripheral progesterone, which is per animal and n is number of animals.

recovered from 6 rabbits treated with cocaine in the binge protocol, and 40 were recovered from 5 saline solution—treated control animals. Although similar numbers of embryos per rabbit were recovered from the two groups, developmental stages were significantly different by the sixth day of in vitro culture (2 × 4 contingency table, $\chi^2 = 14.62$, p < 0.005). More of the embryos from cocaine-treated rabbits were arrested in the one-cell to four-cell stage (p < 0.05) or remained in the morula stage (not significant, p > 0.1), and significantly fewer had reached the blastocyst stage (p < 0.01; Fig. 4) than had those from saline solution—treated rabbits. However, no difference in implantation index between cocaine- and saline solution—treated an-

imals was found on day 8 of pregnancy (Fig. 5). Because implantation in the saline solution group in preliminary experiments was uniformly high and was no different from that observed with any other group (Fig. 5), it was decided that increasing the number of animals in this group was not necessary.

Comment

Most of the animals tolerated both protocols well. However, 4 mg/kg administered at 0.5 ml/min was found to be the highest dose schedule tolerated by this group of female rabbits; increasing the dose to 4.5 mg/kg or the rate of administration to 1.0 ml/min often resulted in severe convulsions. The binge protocol, al-

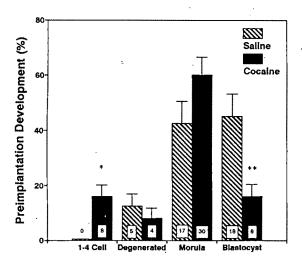


Fig. 4. Embryonic development in vitro assessed on sixth day of culture. Animals were mated and treated with binge protocol. The next day ova and embryos were retrieved and cultured in vitro for 6 days. Fifty embryos were recovered from 6 cocaine-treated rabbits, and 40 were recovered from 5 saline solution-treated control animals. An increased percent of embryos from animals treated with cocaine remained at one-cell to four-cell stage (asterisk, p < 0.05) or morula stage (not significant), and a decreased percent reached blastocyst stage (two asterisks, p < 0.01) as compared with embryos of saline solution-treated controls. Numbers of ova and embroys at each stage are indicated within bars.

though high dose, is within the range seen in compulsive cocaine users who report repeated crack or intravenous cocaine administration at 20- to 40-minute intervals for as long as 24 hours.7

In rabbits, failure to ovulate after mating is probably the result of inadequate luteinizing hormone surge.9 Acute injections of cocaine have been shown to stimulate luteinizing hormone release in rats and monkeys,10,11 whereas repeated cocaine injections before the ovulatory stimulus decrease ovulation in the cycling rat⁵ and mated rabbit⁶ and are associated with decreased serum luteinizing hormone levels.5 These data suggest that the increased ovulation rate observed in rabbits treated with cocaine by the single daily protocol may have been a result of the acute effect of the drug on the hypothalamopituitary unit. The ovulation rates observed in animals treated with saline solution by the single daily protocol or with cocaine or saline solution by the binge protocol and in untreated controls were essentially identical and similar to published ovulation rates for mated rabbits.9

In many species, including humans, cocaine use causes anorexia12 and an attendant reduction in weight or weight gain.13, 14 Some experimenters have found that cocaine treatment during pregnancy reduces fetal or newborn rodent weight,15,16 although others have found that weight is not affected.17, 18 The single daily protocol reduced maternal weight gain in rabbits, but

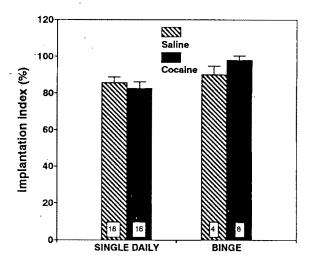


Fig. 5. Implantation index on day 8 of pregnancy in animals subjected to single daily and binge protocols. Implantation index = (Number of implantations/Number of corpora lutea) × 100. There was no difference in implantation index between cocaine and saline solution treatment with either protocol. Number of sides (ovary and ipsilateral uterine horn) examined are indicated within bars.

decreased maternal weight gain did not affect fetal or placental weights. This is not surprising, because restricting maternal food intake to <50% of the minimum requirement only slightly decreases rabbit fetal weight.19 In some drug studies it may be necessary to include a control group that is pair-fed to the same food and water intake, or perhaps weight gain, as the cocaine group. Because the significant differences between cocaine and saline solution groups (ovulation rate, ovulatory efficiency, and effects on subsequent in vitro development when embryos were retrieved on day 1) were seen within 24 hours of starting treatment and before nutritional status would have been affected, there was no need for a pair-fed group in these experiments.

Kaufmann and Savoy-Moore²⁰ found that rabbits injected with cocaine subcutaneously for 5 days before gonadotropin-induced ovulation had lower serum progesterone levels than did saline solution-treated controls. This observation suggests that repeated exposure to cocaine may affect subsequent luteinization. However, we did not observe similar effects in our studies in which the cocaine regimen was begun immediately after mating. Peripheral venous progesterone levels in both cocaine and saline solution groups over the course of pregnancy were similar to those reported by Mikhail et al.21 for untreated rabbits. Average corpora lutea weight during the course of pregnancy was also similar to previously reported values.22 In contrast, ovarian venous progesterone levels in both cocaine- and saline solution-treated groups rose earlier in the pregnancy and remained high longer than did those previously reported for pregnant rabbits.²² It is possible that the daily routine of restraint and injection with cocaine or saline solution increased progesterone secretion into the ovarian vein. Increases in progesterone clearance may have compensated for increased secretion, so that peripheral levels were similar to those seen in normal pregnant rabbits.

The binge protocol exposed follicular oocytes to repeated doses of cocaine between the gonadotropin surge and ovulation, during nuclear and cytoplasmic maturation.²³ Cocaine induces temporary delays in maturation in several systems of the neonatal rat.¹⁶ The decrease in blastocyst development in vitro that we observed with the binge cocaine protocol may have been a similar temporary developmental delay, which may have resulted from cocaine-induced alterations in cytoplasmic maturation. By the time of implantation the previously delayed embryos may have progressed sufficiently so that the implantation of embryos from both cocaine- and saline solution—treated rabbits was similar.

Human cocaine use has been correlated with a decrease in birth weight and an increase in preterm delivery.^{2,3} Is this poor outcome a result of effects of the drug, of the life-style that accompanies use of the drug, or of the interaction between the factors? Although there are dangers in extrapolating observations across species, our results suggest that exposure to the drug alone is not sufficient to account for the poor pregnancy outcome seen in crack users. We found that daily exposure to high levels of cocaine does not reduce fetal weight or result in preterm delivery. It seems likely therefore that the interaction of the drug with accompanying risk factors, such as poor nutrition and a lack of prenatal care, is responsible for the perinatal problems associated with human cocaine use.

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Expression of extracellular matrix—degrading metalloproteinases by cultured human cytotrophoblast cells: Effects of cell adhesion and immunopurification

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In vitro, invasion of basement membrane by human trophoblast can be blocked by metalloproteinase inhibitors. The purpose of our study was to characterize these enzymes by zymography, to define their cellular origin. First-trimester cytotrophoblast cells were prepared according to the method of Kliman et al. Half of the cell suspension was further purified with an antibody to leukocyte common antigen (CD45). Cytotrophoblast cells (immunopurified or not) were incubated in Dulbecco's modified Eagle's medium on different matrices. Progesterone, total human chorionic gonadotropin, and free 8-human chorionic gonadotropin were measured in the supernatant by radioimmunoassay or enzyme immunoassays. Secreted (in the medium) and cell-bound proteases were characterized by zymography on sodium dodecyl sulfate-polyacrylamide gels containing gelatin. Cytotrophoblast cell preparations contained 12% to 34% leukocyte common antigen-positive cells before and 0% after immunopurification. Large zones of digested matrices were observed after 48 hours of culture on Matrigel or rat tail collagen but not on agarose. Cells secreted progesterone, human chorionic gonadotropin, and free β -human chorionic gonadotropin in vitro, but no difference was observed among cells grown on different matrices or between immunopurified and nonimmunopurified cells. By zymography, seven gelatin-degrading enzymes were seen in culture supernatants and five of them were present in cell lysates. The molecular weights of these proteases ranged from 59 to 230 kd. Immunopurification eliminated three of these enzymes, so they were clearly produced by bone marrow-derived cells (leukocyte common antigen positive) contaminating the cytotrophoblast cell preparation. Cells grown on Matrigel express a unique 59 kd gelatinase that was not seen in the supernatants of cells grown on other matrices. Zymography in the presence of inhibitors showed that these enzymes were neutral metalloproteinases, which might be responsible for the observed extracellular matrix degradation. (Am J OBSTET GYNECOL 1991;165:1791-1801.)

Key words: Cytotrophoblast, metalloproteinases, immunopurification, extracellular matrix

The hypothesis that the invasiveness of trophoblast is associated with its proteolytic activity was first proposed by Mossman¹ >50 years ago. Experimental evidence relating trophoblast invasiveness and proteolysis was produced much later by Blandau,² who showed that guinea pig trophoblast was capable of digesting a dried gelatin film. The same observation was also made for the rabbit blastocyst.³ The study of the role of proteinases in trophoblast implantation effectively began with the publication of Strickland et al.,⁴ who showed that the trophectoderm of mouse blastocyst produced plasminogen activator, a well-known serine protease, and

that the maximal rate of production of this enzyme was observed at the time of implantation. The importance of plasminogen activator in implantation was further documented in mutant mice with implantation deficiencies. The blastocysts of these mice attached and grew in vitro but showed a decreased invasiveness that correlated with a decreased plasminogen activator production.⁵ Human trophoblast also produces plasminogen activator.⁶

Although plasminogen activator plays a significant role in trophoblastic invasiveness, it is probably not the only protease involved in this process. Mouse blastocysts cultured on a radiolabeled matrix release radioactive peptides. This proteolytic activity seems not to be due to plasminogen activator, because digestion of the matrix still occurs in cultures depleted of plasminogen. Furthermore, in the presence of plasminogen and of ε-aminocaproic acid, a specific inhibitor of plasminogen activator, degradation of the extracellular matrix remains visible. Yagel et al. also showed that ε-aminocaproic acid blocked the invasion of basement membrane by human trophoblast. In the same study

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Table I. Immunocytochemistry of cytotrophoblast cells in culture (range, n = 3)

Antibody used	Specificity	Before purification (%)	After purification (%)
β-hCG	Trophoblast	0-8	ND
Human placental lactogen	Trophoblast	0-10	. ND
Pregnancy-specific β-gly- coprotein	Trophoblast	5-11	ND
HLA class I	Extravillous tro- phoblast	30-43	ND
Keratin	Epithelial origin	50-72	90-97
LCA	Lymphomyeloid	12-34	0
Factor VIII	Endotheliál	0	0
Vimentin	Fetal stroma and endothelial cells and lym- phomyeloid	2-5	3-7

ND, Not determined.

metalloprotease inhibitors had the same effect as ε-aminocaproic acid, indicating that, besides plasmin-plasminogen activator, metalloproteases might also be important in controlling trophoblastic invasion. The same conclusion was reached in a very recent study by Librach et al., who showed that antibodies to plasminogen activator block only partially the invasion of cytotrophoblast cells into Matrigel, whereas antibodies to the 92 kd collagenase inhibit this invasion completely.

Fisher et al.10 demonstrated the presence of several metalloproteinases secreted by first-trimester cytotrophoblasts, some of which were absent or greatly reduced later in gestation. Unfortunately, bone marrowderived cells, which contaminate cytotrophoblast cell preparations and which are known to release proteases, were not eliminated by their cell purification technique. It is the purpose of our study to define more precisely the origin and nature of the proteases secreted by cytotrophoblast cell preparations. To this end care has been taken to immunopurify cytotrophoblast cells and to discard those cells of lymphomyeloid origin. We also investigated the role of cell adhesion in the induction of protease secretion and cultured immunopurified trophoblast cells on different substrata and extracellular matrices to investigate the changes in their morphologic features and secretion.

Material and methods

Preparation of trophoblast cells. First-trimester trophoblastic tissue was obtained from legal abortions between 6 and 12 weeks of gestation. All manipulations were performed under sterile conditions in a laminar flow hood. Within 15 minutes from vacuum aspiration, the products of conception were washed with sterile phosphate-buffered saline solution and the trophoblast was separated manually from other tissues under a stereomicroscope. This tissue was minced and washed twice with Hanks' balanced salt solution (Sigma, St. Louis) containing 200 U/ml penicillin and 200 µg/ml streptomycin (Hoechst, Darmstadt). The washed tissue was

then incubated at 37° C five times for 10 minutes in Hanks' balanced salt solution containing 0.25% trypsin (Difco, Detroit), antibiotics, 2.5 µg/ml amphotericin B (Gibco, Basel), 100 μg/ml gentamicin (Gibco), 500 U/ml deoxyribonuclease (Sigma), 1 mol/L magnesium sulfate (Fluka, Buchs, Switzerland), and 25 mmol/L N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (Hepes) (Boehringer, Mannheim, Germany). After each digestion step, the supernatant was neutralized by addition of 10% fetal bovine serum (Amimed, Birsfelden, Switzerland). The supernatants were pooled, centrifuged at 800g for 10 minutes, resuspended in the same medium without fetal bovine serum, and filtered over nylon (mesh size 100 µm). The filtered cell suspension was then submitted to a 5% to 70% discontinuous Percoll gradient according to Kliman et al.11 The fraction containing the cytotrophoblast cells (densities 1.051 to 1.065) was washed with Dulbecco's modified Eagle's medium (Gibco) containing 10% decomplemented and acid-treated fetal bovine serum. Acidtreated fetal bovine serum was obtained after decomplementation (56° C for 30 minutes) by lowering the pH to 3 with hydrochloric acid (1 mol/L) for 2 hours at room temperature and bringing it up again at pH 7.4 with sodium hydroxide (1 mol/L). The washed cells were counted in a Neubauer cell, and half the cells were incubated (1 million per milliliter) in Dulbecco's modified Eagle's medium containing 2 mmol/L L-glutamine (Gibco), 4.2 mmol/L magnesium sulfate, 25 mmol/L Hepes, 10% decomplemented and acidtreated fetal bovine serum, 1% gentamicin, 1% amphotericin B, 100 μg/ml streptomycin, and 100 U/ml penicillin (culture medium). The other half were immunopurified.

Immunopurification of cytotrophoblast cells. The washed cytotrophoblast cells (5 million in 2 ml) were incubated for 30 minutes at 4° C with 30 µl of a monoclonal antibody to leukocyte common antigen (LCA) (Dakopatts, Copenhagen). After incubation, the cells were washed with phosphate-buffered saline solution

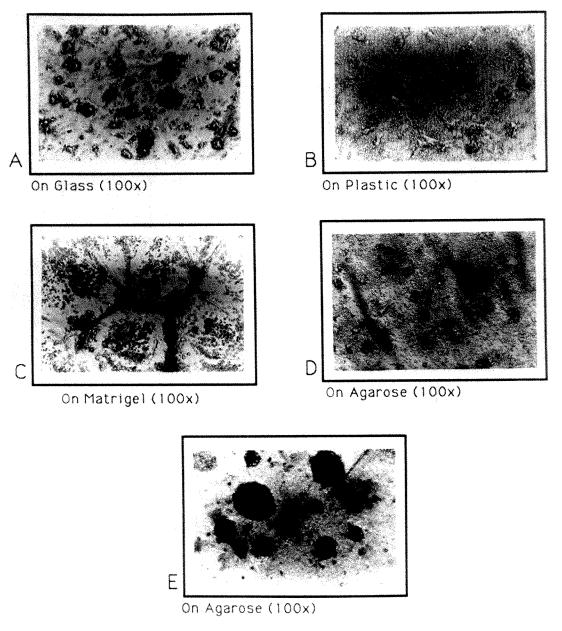
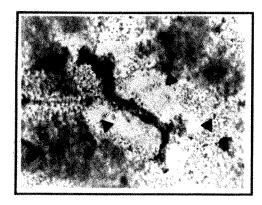


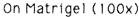
Fig. 1. Purified cytotrophoblast cells grown on glass (A), plastic (B), Matrigel (C), and agarose (D) for 3 days. E, Same as D but after 7 days of culture.

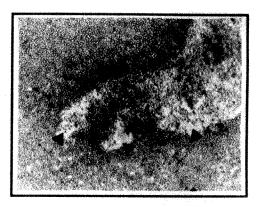
containing 0.1% bovine serum albumin (radioimmunoassay grade, Sigma). Prewashed (with phosphatebuffered saline solution and bovine serum albumin) magnetic particles coated with a second antibody (30 µl Dynabeads, Dynal, Milian Analytica, Switzerland) were then incubated with the cell suspension for 20 minutes at 4° C. A magnet was applied along the test tube to retain the particles bound to the cells expressing LCA on their surfaces. The supernatant was poured into a clean test tube, and the immunopurified cells were counted in an Neubauer cell and diluted to 1 million cells per milliliter in the culture medium.

Cell culture. Two hundred microliters of cytotro-

phoblast cells or immunopurified cytotrophoblast cells (1 million per milliliter culture medium) was incubated in 12-well tissue culture plates (Costar, Cambridge, Mass.) under a 5% carbon dioxide and 95% air atmosphere in an incubator at 37° C. The wells were precoated with 300 µl Matrigel per well (Collaborative Research, Inotech, Switzerland), 300 µl 0.5% agarose in phosphate-buffered saline solution (Agar Noble, Difco), or 300 µl rat-tail collagen (prepared as described previously).12 Uncoated wells or wells containing glass slides were also used. Every other day the cells floating in the medium were carefully returned to the wells after centrifugation, and the supernatant was divided into aliquots and immediately frozen at -20° C. At the end







On Collagen (100x)

Fig. 2. Cultures of purified cytotrophoblast cells grown on Matrigel or rat tail collagen after 7 days. Note large zones of digested matrices (arrowheads).

of the culture period (8 to 10 days), the cells were washed and lysed with 200 μ l Triton X-100 (2.5% in water) and then stored at -20° C until assayed.

Hormone assays. Total human chorionic gonadotropin (hCG) was measured in the culture supernatant with a microparticle enzyme immunoassay with a sensitivity of 1 mIU and a coefficient of variation of 3.6% (Abbott, Abbott Park, Ill.). Progesterone and free β-hCG were measured by commercially available radioimmunoassay kits (CIS, sensitivity 0.25 ng/ml, coefficient of variation 7.3%; Mediginex, sensitivity 0.04 ng/ml, coefficient of variation 5.3%, Medipro AG, Teufen, Switzerland). Results were expressed per 1 million cells as milli—international units or nanograms per milliliter.

Zymography. Proteases were visualized by zymography with a modification of the technique described by Fisher et al. 10 Briefly, 10% polyacrylamide gel in 0.1%sodium dodecyl sulfate containing 1 mg/ml gelatin (Merck, Darmstadt, Germany) or casein (Sigma) were cast at a final dimension of $80 \times 85 \times 0.8$ mm. After polymerization of the 10% gel, a 2% polyacrylamide stacking gel was cast above the previous one. Molecular weight standards (Pharmacia, Uppsala) and samples to be analyzed (30 µl) were incubated at room temperature for 5 minutes with 5 µl sample buffer (17.4% sodium dodecyl sulfate, 7% sucrose, and phenol red in water). Twenty-five microliters of the incubate was placed over the stacking gel and electrophoresis was run at 7 A for approximately 5 hours at 8° to 10° C in 25 mmol/L Tris hydrochloride containing 0.19 mol/L glycine and 0.1% sodium dodecyl sulfate, pH 8.6.

After electrophoresis, the gel was put through six 5-minute washes in 2.5% Triton X-100 (2.5% in water) and three times for 5 minutes in phosphate-buffered saline solution. The gel was then placed in phosphate-buffered saline solution, pH 7.4, containing 0.9 mmol/L calcium chloride and magnesium chloride (in

the presence or absence of protease inhibitors) and incubated overnight at room temperature on a moving platform. The next morning the gel was stained with Coomassie Brilliant Blue G250 (0.1% in 25% methanol and 10% acetic acid in water, Fluka) and destained in 5% methanol and 7.5% acetic acid in water.

Protease inhibitors used were 1 mmol/L ethylene-diaminetetraacetic acid (Fluka), 0.2 mmol/L 1,10-phenanthroline, 2 mmol/L phenylmethylsulfonylfluoride, 1 mmol/L pepstatin A, and 1 mmol/L iodoacetamide (all from Sigma).

Immunocytochemistry. Cells were centrifuged at 700g onto glass slides with a Cytospin centrifuge (IG, Geneva). They were fixed 5 minutes in acetone at -20° C, and the slides were washed twice with phosphate-buffered saline solution. Fifty microliters of phosphate-buffered saline solution and bovine serum albumin (5%) was added to the cells and incubated for 10 minutes at room temperature. The phosphate-buffered saline solution and bovine serum albumin drop was gently blotted and replaced by 50 µl of the first antibody (see text that follows). After 30 minutes the glass slides were washed twice in phosphate-buffered saline solution and the alkaline phosphatase-second antibody complex (50 µl immunoglobulin G rabbit antimouse, Dako) was added to the cells. After a 30-minute incubation the second antibody was replaced by a third antibody (alkaline phosphatase coupled with antirabbit immunoglobulin G, 50 µl, Dako) and incubated as described in the preceding text. The slides were then stained with naphthol AS-BI phosphate (Sigma), 5 mg in 50 µl N,N-dimethylformamide containing 10 mg Fast Red and 10 mg levamisole in Tris hydrochloride, 0.1 mol/L, pH 8.2 After a 5-minute incubation in a moist atmosphere, the slides were washed and counterstained with hematoxylin. The slides were evaluated under the microscope, and the results were expressed

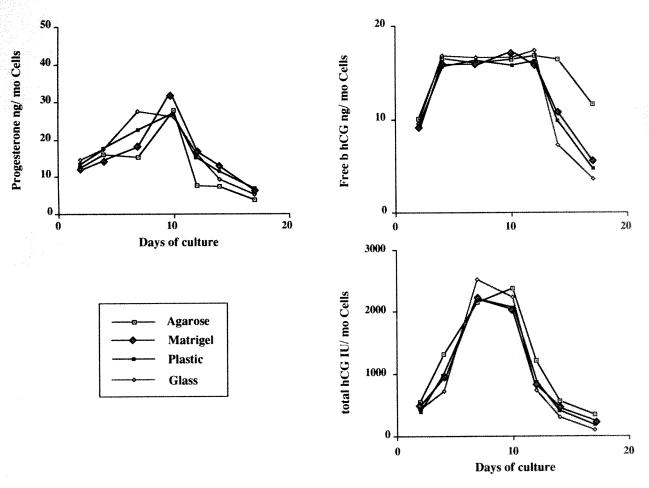


Fig. 3. Effect of matrices on hormone production by purified cytotrophoblast cells.

as the percent of positive cells per 300 total cells counted.

The first antibodies used included pregnancy-specific β₁-glycoprotein (1:50), hCG (1:100), placental lactogen (1:20), human leukocyte antigen (HLA) class I (1:50), vimentin (1:20), LCA (1:20), and factor VIII (1:20) (all from Dako), and anticytokeratin (clones AE1 and AE3, 1:50, Boehringer; clones CAM 5 and 2, Becton-Dickinson, Inotech). These last two antibodies were used as a 1:1 mixture. Negative controls were run in parallel and were obtained by replacing the first antibody with phosphate-buffered saline solution containing 5% bovine serum albumin.

Results

Immunocytochemistry. As shown in Table I, hCG-, pregnancy-specific β₁-glycoprotein-, and placental lactogen-positive mononuclear cytotrophoblast cells represented <10% of the total cell population. More than one third of the cells in the cell suspension were positive for HLA Class I antigens, indicating either an extravillous origin of trophoblastic cells or the presence of lymphomyeloid cells.

Before immunopurification only 50% to 72% of the cells were epithelial in origin and keratin positive, whereas after purification these values increased to 90% to 97%. Lymphomyeloid cells accounted for 12%to 34% before purification but became undetectable after immunopurification. No endothelial cells could be seen either before or after purification (0%, factor VIII), whereas purification did not affect the low contamination by fetal stromal cells (2% to 7% vimentin positive). Thus after immunopurification the cell suspension represented 90% to 97% cytotrophoblastic cells with a 3% to 7% contamination by fetal stromal cells (Table I).

Behavior of cells in culture. Within 12 hours of culture, mononuclear cytotrophoblast cells adhered to glass, plastic, Matrigel, or rat-tail collagen but not to agarose. The cells flattened out on these matrices and grew as small colonies of aggregated cells (Fig. 1, A and B), but on Matrigel the cells aggregated in a netlike pattern with large zones devoid of cells in the immediate vicinity of the aggregates (Fig. 1, C). After 72 hours of culture, syncytium-like structures formed on all matrices except agarose. On agarose, cells did not

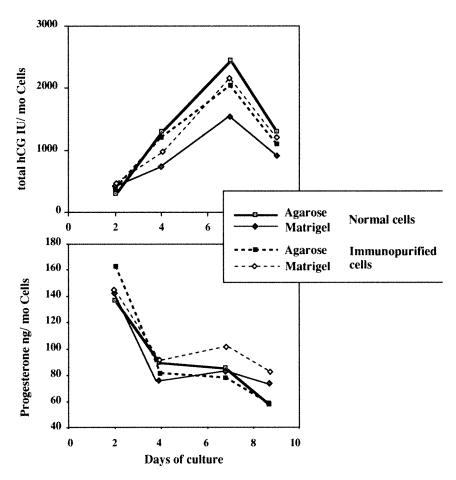


Fig. 4. Effect of immunopurification on hormone production by cultures of cytotrophoblast cells.

attach to the substrate and remained floating in the medium, where they grew as aggregates. With time the size of these aggregates increased considerably, with no evidence of attachment (Fig. 1, D and E). On both rattail collagen and Matrigel, large zones devoid of cells could be seen clearly after 3 to 4 days in culture. After the matrices in the wells were stained with Coomassie blue, the cell-free zones did not stain, showing a complete digestion of the matrix in these areas (Fig. 2, A and B).

Hormone production. Immunopurified cytotrophoblast cells (10^6 cells/ml) were cultured up to 17 days on the different matrices, and the medium was changed every other day. As shown in Fig. 3, the nature of the matrix on which the cells were cultured did not change the secretion pattern of progesterone and total or free β-hCG. Irrespective of the culture conditions, the concentrations of progesterone and total hCG increased from day 0 to day 10 and gradually decreased thereafter. The concentration of free β-hCG followed a different pattern. A plateau was reached by the fourth day of culture and lasted up to day 12 before decreasing. Here also, adhesion of the cells to the matrix (as

in glass, plastic, or Matrigel), as compared with cells that did not adhere to the matrix (as in agarose), had no effect on free β -hCG secretion. Immunopurification per se did not change the secretion of total hCG or progesterone by cytotrophoblast cells cultured on agarose or Matrigel (Fig. 4).

Protease production. Gelatin zymography, but not casein zymography, of cytotrophoblast cell supernatants after 48 hours of culture revealed seven bands of digestion ranging from 59 to 230 kd (Fig. 5); three of them (230, 94, and 64 kd) were particularly intense, whereas the other ones (197, 128, 73, and 59 kd) appeared much weaker. Only four of these seven bands (230, 128, 94, and 65 kd) were associated with the cells, because they were expressed in the zymograms of the cell lysates (Fig. 6). Two other gelatinases (197 and 59 kd) appeared only to be secreted, because they were not visible in the zymogram of the cell lysate; band 5 (73 kd) was present in the medium incubated for 48 hours in the absence of cells (Fig. 5, lane 2; Fig. 6, bottom, lane 5) and probably represented a contaminant. The 230 and 128 kd gelatinases are clearly of lymphomyeloid origin, because these bands were no

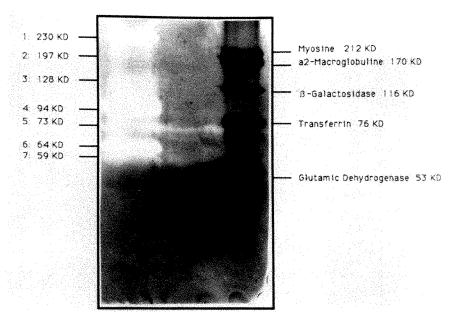


Fig. 5. Zymogram of supernatant of purified cytotrophoblast cells grown for 48 hours on Matrigel (first lane); cell-free incubation medium after 48 hours of incubation on Matrigel (second lane), and standards (third lane).

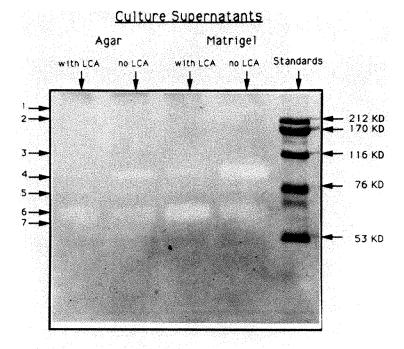
longer visible after immunopurification of the cytotrophoblast cells (Figs. 6 and 7). Immunopurification reduced the intensity of the 94 kd gelatinase, indicating its dual origin: trophoblastic and lymphomyeloid (Figs. 6 and 7), whereas the 64 kd gelatinase seemed more active after immunopurification (Figs. 6 and 7). Thus one can consider that mononuclear cytotrophoblast cells release four unique gelatinases of 197, 94, 64, and 59 kd. When compared with cells grown on agarose or plastic, cells grown on Matrigel seemed to express the 94 kd gelatinase more intensely (Fig. 7), whereas the other proteinases remained unchanged. However, this was true only when the cells were not immunopurified (Fig. 7), perhaps indicating that Matrigel might specifically activate the 94 kd gelatinase of lymphomyeloid origin. The 59 kd proteinase appeared to be induced only by collagen because it was present in the supernatants of cells grown on Matrigel and rat-tail collagen but absent when the cells were grown on agarose or plastic (Fig. 7). The pattern of expression of gelatinases secreted in the supernatants of cells grown on glass was superimposable to the pattern of cells grown on plastic, and those grown on rat-tail collagen were similar to the patterns of cells grown on Matrigel (results not shown).

As shown on Fig. 7, when the gels were incubated overnight with phosphate-buffered saline solution containing 1 mmol/L ethylenediaminetetraacetic acid, digestion bands were no longer visible. The same observation was made when ethylenediaminetetraacetic acid was replaced by 1:10 phenanthroline (results not shown). No inhibition was observed with pepstatin A or iodoacetamide. In contrast, a complete inhibition of the gelatinases was observed when divalent ions (calcium and magnesium) were absent from the gel incubation buffer.

Comment

Human placental cells isolated by enzymatic digestion and cultured in vitro are known to yield a mixed cell population.13 Contaminating cells include macrophages, fibroblasts, endothelial cells, and blood elements. Several attempts to improve the purity of trophoblast cell cultures have been proposed on the basis of physical properties,11 immunologic properties,14 or biologic properties.^{15, 16} One such technique of immunomagnetic purification was using HLA class I and II antibodies14 to remove contaminating cells (mostly macrophages and fetal stroma). Our purpose of characterizing the proteases secreted by cytotrophoblast cells prompted us to develop a technique that unequivocally eliminated known sources of extratrophoblastic proteases, such as monocytes, macrophages, and other lymphomyeloid cells.

Because extravillous cytotrophoblast cells in culture express HLA class I antigen,15 the use of an anti-HLA antibody will eliminate those cells together with those of lymphomyeloid origin. Extravillous cytotrophoblast cells represent the cells invading the uterine lining,15 thus the cells that potentially express the proteases. These considerations guided our choice in using an



Cell Lysates

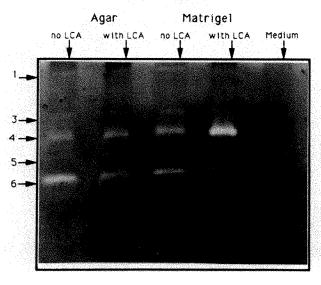


Fig. 6. Zymograms of supernatant and cell lysates of cytotrophoblast cells purified (*with LCA*) or not (*no LCA*) grown on agarose or Matrigel.

anti-LCA antibody to eliminate only lymphomyeloid cells. The technique allowed >90% isolation of cytotrophoblast cells; however, this choice had its drawbacks because fetal stromal cells still contaminated our cultures by about 5%. Very recently and independently, Librach et al. used the same antibody with a similar technique to purify their cytotrophoblast culture; they reported corresponding success.

The phenotype of the cytotrophoblast cells observed in our study corresponded to that of previous descriptions, $^{14.17\cdot19}$ with the exception that adhesion of cells to glass or plastic was not as extensive as described because only about 50% to 70% of the cells adhered. It is not known if this was due to the use of acid-treated fetal bovine serum.

Aggregates or syncytia were clearly visible on glass, plastic, collagen, and Matrigel. In contrast, no adhesion was seen on agarose where spheroid-like aggregates formed in the supernatant. No attempts were made in this study to define whether aggregates represented

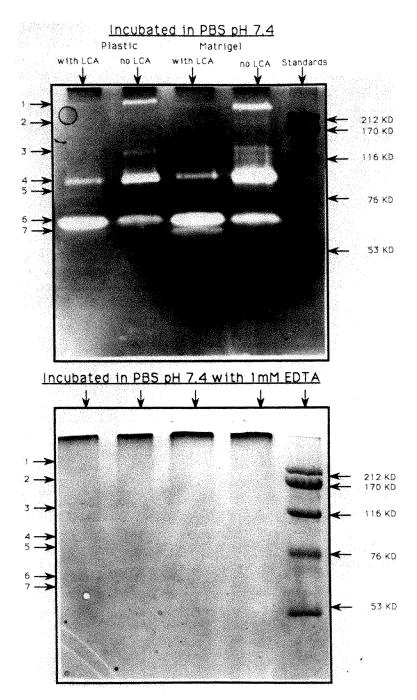


Fig. 7. Zymograms of supernatants of cytotrophoblast cells purified (with LCA) or not (no LCA) grown on Matrigel or plastic. Gels were incubated without (upper panel) or with (lower panel) ethylenediaminetetraacetic acid.

true syncytia or merely aggregated cells, because the cell boundaries were not easily visible under the inverted microscope and staining for desmoplakin as recommended18 was unsuccessful in our hands.

In agreement with other studies,20,21 the mononuclear cytotrophoblast cells produced hCG and progesterone with the pattern of production changing rapidly with the age of the placenta. This is illustrated by the differences in the secretion profiles of progesterone and hCG between Figs. 3 and 4 where 11- and 7-weekold placentas were used, respectively. The secretion of free β-subunit followed a slightly different pattern than hCG with a constant production over a period of 10 days, a finding not reported before. We observed, as already reported,22 that adhesion in itself and the nature of the matrix did not influence the endocrine activity of cytotrophoblast cells. Because adhesion of the cytotrophoblast cells seems to be a prerequisite for syncytium formation, our observation would imply that biochemical and morphologic differentiation can be uncoupled, an observation previously made by others. 22 In addition to the cells' endocrine function, the presence of four unique extracellular matrix-degrading metalloproteinases produced by cytotrophoblast cells and identified by zymography was reported by us. The molecular weights of these proteinases were approximate because their determination is influenced by the choice of markers, acrylamide concentration, and copolymerized substrate. The metalloproteinases described here correspond to those described by Fisher et al.,10 who demonstrated the presence of five metalloproteinases (68 to >200 kd) secreted by first-trimester trophoblast cells. However, in their culture system the lymphomyeloid cells were not eliminated. In a very recent article from the same group9 they also used an LCA-based immunopurification technique but did not show any zymograms. Although the four cytotrophoblast-specific metalloproteinases (197, 94, 64, and 59 kd) described here were not influenced by adhesion of the cells to the substrate, depending on the nature of the substrate, regulatory effects were seen. The 59 kd gelatinases were observed only when cells were cultured on substrata containing type I collagen, and the 94 kd gelatinase was much more expressed when the cells were grown on Matrigel. This last observation was true only for nonimmunopurified cells, indicating either that Matrigel specifically stimulates the 94 kd gelatinase of bone marrow-derived cells or that a matrigel-induced paracrine factor of cytotrophoblastic origin regulates the nontrophoblastic enzyme. That some of the described proteases were active in the culture is indicated by the fact that the cells degraded both matrigel and collagen, an observation previously made by others. 9, 10, 19 This degradative behavior seems to be due essentially to the 92 kd collagenase.9 The fact that the gelatinases were active in culture is of importance because proteases are secreted as proenzymes and sodium dodecyl sulfate (used for zymography) is known to artifactually activate the proenzymes.

Although the nature of the described cytotrophoblastic metalloproteinases has not been defined, it is interesting to note that neutral metalloproteinases of the same molecular weights have been described in other cell cultures. Corneal fibroblasts,²³ alveolar macrophages,²⁴ and embryonic cells²⁵ all express a 92, 67, and 51 kd neutral metalloprotease recognized as the 92 kd collagenase type IV, the 72 kd collagenase (which migrates at 64 to 67 kd in copolymerized gels), and stromelysin (faint band on gelatin at 51 kd), respec-

tively. These enzymes belong to the so-called matrix metalloproteinase family. These proteases are classified as MMP-1, that cleaves collagen types I, II, and III, called interstitial collagenases (52, 64 kd); MMP-2 or gelatinases, that degrade collagen types IV and V (92 kd) and collagen types IV, V, VII, and fibronectin (67 kd); and MMP-3, called proteoglycanases or stromelysins which cleave proteoglycans, type IV collagen, casein, and fibronectin (51 kd). More recently, similar matrix metalloproteinases have been described in hepatocytes²⁶ and human sertoli cells.²⁷ Thus cytotrophoblasts express an array of matrix metalloproteinases similar to many other cells. But in contrast to these other cells, cytotrophoblasts have an invasive phenotype. Taking into account the capacity of matrix metalloproteinases to digest basement membrane and extracellular matrix components, one must suppose that the regulation of activity is a phenomenon specific to each cell type.

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Improvement of in vitro fertilization and early embryo development in mice by coculture with human fallopian tube epithelium

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Coculturing one- and two-cell embryos with various cell lines has been shown to overcome species-specific developmental blocks and to improve blastocyst transformation rates. The objective of this study was to assess whether human fallopian tube epithelium organ explants influence in vitro fertilization and subsequent early embryo development in a murine model. Fertilization, blastocyst transformation, and blastocyst expansion and hatching rates were significantly higher in the coculture group when compared with rates for culture in standard media or media conditioned by human tubal explant cultures. The results from conditioned and unconditioned media were not significantly different. (AM J OBSTET GYNECOL 1991;165:1802-5.)

Key words: Fallopian tube, coculture, in vitro fertilization, embryo development, mouse

The birth of the first baby conceived by means of in vitro fertilization and embryo transfer established that the fallopian tube was not essential for reproduction. As a result research interest in the fallopian tube waned as shown by a reduction in the number of manuscripts in the clinical literature. During this time much attention was focused on methods of improving the low pregnancy rates achieved with in vitro fertilization and embryo transfer. In spite of an enormous volume of published literature resulting from these efforts and more than a decade of clinical experience worldwide, the pregnancy rates remain disappointing.

In addition to its transport function, the fallopian tube provides a unique environment for sperm capacitation, fertilization, and early embryo development. Because this environment is very poorly characterized, it cannot be artificially duplicated in vitro. Animal studies have revealed that preimplantation embryo development in vitro is delayed when compared with development in vivo, and culturing embryos in vitro is hampered by species-specific "blocks" to development. Also, clinical pregnancy rates with in vitro fertilization and embryo transfer are generally lower than those of gamete and zygote intrafallopian transfer, which deliver the gametes or early preimplantation embryos, respectively, into the fallopian tubes. These obser-

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vations suggest that the fallopian tube contributes something that is missing in vitro.

Studies with several mammalian species have shown that early embryo development is improved by coculture with various cell lines: fibroblasts, Vero cells, cumulus cells, trophoblastic vesicles, and oviductal epithelium. Studies comparing coculture of the oviduct with that of other cell lines demonstrated the superiority of the oviduct; this implies a tissue-specific benefit. Coculturing human preimplantation embryos with fibroblasts, S. E. Vero cells, To and fallopian tube epithelial cell suspensions that of the standard culture system.

Those studies consistently documented facilitated in vitro development of early preimplantation embryos with coculture. There are no studies to date that analyze whether coculture also may increase fertilization rates in vitro. As noted, human tubal epithelial cell suspension cocultures augmented human development of pronuclear embryos to the blastocyst stage. Human fallopian tube epithelial monolayers also yielded significantly higher blastocyst rates from two-cell mouse embryos than media alone. No data are available regarding the utility of human fallopian tube epithelial organ explants in coculture. This study was designed to address these questions by means of a murine in vitro fertilization model.

Material and methods

Fallopian tube culture. Human fallopian tube epithelial organ explants were established as previously described.¹³ The ampullary segments of normal fallopian tubes were obtained at the time of hysterectomy

Table I. Fertilization, blastocyst transformation, and blastocyst expansion-hatching rates from two-cell embryos

	Fertilization	Blastocyst transformation	Expanded or hatched	
Control Conditioned	70.5 ± 8.5 70.5 ± 9.3	29.5 ± 11.6 27.4 ± 8.5	4.4 ± 4.6 9.7 ± 10.2	
Coculture	$81.2 \pm 5.9*$	$58.2 \pm 13.4 \dagger$	$40.8 \pm 17.0 \dagger$	

Data represent mean percentages \pm SD (n = 13).

for benign gynecologic indications in women of reproductive age. The specimens were transported to the laboratory in Dulbecco's modified Eagle's medium (Gibco, Grand Island, N.Y.) at 4° C. The cultures were initiated within 1 hour after excision. The tubes were opened longitudinally with fine dissecting scissors. Strips of the luminal epithelium were excised with scissors and sectioned into segments of 3 mm². These were then washed three times in phosphate-buffered saline solution (Irvine Scientific, Santa Ana, Calif.) before being placed into plastic Petri dishes that were 9 cm in diameter with 5 ml of high-glucose Dulbecco's modified Eagle's medium (25 mmol/L glucose) supplemented with 2.5% fetal bovine serum (Gibco), penicillin, and streptomycin. There were approximately 20 to 25 explants per dish. The epithelial nature and viability of the tissue were confirmed by the observation of vigorous ciliary beating by means of phase-contrast microscopy at culture initiation and at each change of media. The cultures were maintained in a 37° C incubator with 5% carbon dioxide and 95% humidity. The medium was replaced daily. Conditioned medium was collected at the time of change. Explants and conditioned media were used within 7 days after the culture was established.

Murine in vitro fertilization. Female CB6F1 mice (Charles River, Wilmington, Mass.) that were between 6 and 8 weeks old were induced to superovulate with 7.5 IU pregnant mare serum gonadotropin administered intraperitoneally; this was followed by the intraperitoneal administration of 7.5 IU human chorionic gonadotropin 48 hours later. The mice were killed by cervical dislocation 15 hours after human chorionic gonadotropin administration, and the oviducts were excised. The cumulus masses were dissected from the oviducts with tuberculin needles in 2.5 ml phosphatebuffered saline solution. The cumulus masses were pooled in high-glucose Dulbecco's modified Eagle's medium with 1% bovine serum albumin (insemination media, Irvine Scientific) and were then randomly allocated to the coculture group or to the conditioned or unconditioned media group.

Mature CB6F1 male mice were killed by cervical dislocation, and both epididymides were excised. Sperm was obtained by compressing the epididymides in 0.5

ml in insemination medium, and a motile sperm concentration was determined with a Makler chamber (Sefi-Medical Instruments, Ltd., Haifa). The sperm was incubated for 1 hour at 37° C before insemination. Insemination was performed with approximately 500,000 motile sperm in a center well dish with approximately 20 oocytes in 1 ml of insemination medium. A single epithelial explant was placed with the gametes in the coculture group. The conditioned media group consisted of a 1:1 mixture of conditioned medium and unconditioned medium. The dishes were maintained in an incubator at 37° C with 5% carbon dioxide. Afer 6 hours the oocytes were transferred to high-glucose Dulbecco's modified Eagle's medium with 0.5% bovine serum albumin (growth medium). The epithelial explant was transferred with the oocytes in the coculture group. Half the medium in this group was replaced every 48 hours. The oocytes or embryos were not in physical contact with the explants. In the conditioned medium group, growth medium was mixed 1:1 with conditioned medium.

The oocytes were observed under a dissecting microscope every 24 hours for the 5 days after insemination. Fertilization was defined as cell division 24 and 48 hours after insemination. Blastocyst transformation, expansion, and hatching were calculated from the twocell stage. Statistical analysis was performed by analysis of variance with repeated measures; the Student-Newman-Keuls test was used for post hoc comparisons.

Results

Thirteen trials were run in duplicate with 1528 oocytes: 505 in the control group, 472 in the conditioned medium group, and 551 in the coculture group. All fallopian tube specimens were obtained during the proliferative phase as confirmed by endometrial histologic studies. Fertilization and embryo development rates are shown in Table I. Fertilization rates were significantly higher in the coculture group than in the control or conditioned medium groups, which had identical rates. Blastocyst transformation and subsequent full expansion and hatching rates were very significantly increased in the coculture group. The differences between the control and conditioned medium groups were not statistically significant.

^{*}p = 0.002.

[†]p < 0.000001.

Comment

Our data are consistent with those in the published literature that report improved early embryo development in coculture as a benefit that is not species specific. This is the first study to assess human fallopian tube epithelial organ explants in coculture. Human fallopian tube epithelial monolayers and cell suspensions in coculture have been shown to be effective, in spite of the fact that they lack ciliated cells. 11, 12, 14, 15 Also, there are no data regarding whether they are capable of secretory function in vitro. In contrast, secretory function has been documented in human tubal explants, 16, 17 which maintain their normal histologic architecture in addition to vigorous ciliary beating.13 Also the method used to establish the explant cultures is much simpler than that for epithelial monolayers and cell suspensions.

We found no differences between the conditioned medium and control groups. Conditioned medium from fibroblasts18, 19 or oviductal monolayers6, 20, 21 did not improve embryo development, although coculture did. It was suggested that cell-to-embryo contact enhances embryo development in vitro.19 In our study there was no physical contact between the oocytes-embryos and the explants. The beneficial effects of the coculture may result from the removal of a detrimental factor or factors from the medium, an alteration in oxygen tension, and/or the secretion of a labile product that does not retain its activity in the conditioned medium. In other studies, however, conditioned medium from oviductal monolayers12, 22 and from trophoblastic vesicles28 did yield improved embryo development rates, which suggests that the effect may have been mediated by a metabolite or secretory product.

In the almost 30-year history of the use of embryo coculture, the possibility of improving fertilization rates in vitro by coculture has not been previously investigated. Our findings confirming this hypothesis have obvious potential applications to the animal husbandry industry, as well as to clinical in vitro fertilization and embryo transfer. Because we obtained the oocytes from the oviducts, it is possible that the higher fertilization rate in vitro with coculture was due to enhanced sperm function. Recently, it has been shown that sperm motility and fertilizing capacity may be maintained and that hyperactivated motility may be induced by the endosalpingeal epithelium.24 Lippes and Wagh25 reported that fallopian tube glycoproteins bind to human sperm. It may be anticipated that results with clinical in vitro fertilization and embryo transfer could be even more impressive, because this method would use follicular oocytes that have not had the potential benefit of residing within the fallopian tubes. Studies with several mammalian species demonstrated that oocytes incorporate oviductal glycoproteins.26-29 Yang and

Yanagimachi²⁹ reported that hamster oviductal oocytes were physically altered when compared with ovarian oocytes. The former had a greater percentage of acrosomal sperm on the zona surface and were more rapidly penetrated by sperm. Also, in studies in which cocultured embryos were transferred to pseudopregnant recipients, higher implantation and pregnancy rates were noted. As 9 We hope that the apparent disparity between in vitro fertilization and embryo transfer and gamete intrafallopian transfer pregnancy rates can be reduced by more closely simulating the fallopian tube environment in vitro with the addition of a tubal epithelial explant to the culture system.

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Comparison of intermittent and continuous use of a gonadotropin-releasing hormone antagonist (Nal-Glu) in in vitro fertilization cycles: A preliminary report

Denise L. Cassidenti, MD, Mark V. Sauer, MD, Richard J. Paulson, MD, Edward C. Ditkoff, MD, Jean Rivier, PhD, Samuel S.C. Yen, MD, DSc, and Rogerio A. Lobo, MD

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The agonistic effect of the gonadotropin-releasing hormone agonist often necessitates an extended period of treatment, resulting in a longer treatment cycle and increased cost. We have evaluated the intermittent use of a gonadotropin-releasing hormone antagonist, Nal-Glu, and have designed a new, simplified protocol for its use in in vitro fertilization. Seven women who had previously undergone treatment with leuprolide acetate and human menopausal gonadotropins were treated with Nal-Glu. Leuprolide acetate, 1 mg/day subcutaneously, was administered in the midluteal phase until down regulation was achieved (estradiol <30 pg/ml). Human menopausal gonadotropins, three to four ampules per day intramuscularly, was administered in conjunction with 500 µg subcutaneous leuprolide acetate. In the treatment cycles Nal-Glu (50 µg/kg/day) was administered intramuscularly on cycle day 1 or 2 for 3 days to achieve down regulation. Human menopausal gonadotropins, three to four ampules intramuscularly, was then administered daily without the antagonist. Nal-Glu was resumed when the follicles reached 14 to 16 mm and was continued until the day of human chorionic gonadotropin administration. Compared with leuprolide acetate-human menopausal gonadotropins cycles, the days required for down-regulation with Nal-Glu were significantly shortened (20.6 \pm 4.1 vs 1.6 \pm 0.3 days, p < 0.001), as was total cycle length $(31.3 \pm 5.8 \text{ vs } 11.0 \pm 1.0 \text{ days}, p < 0.01)$. The mean number of days of treatment with human menopausal gonadotropins, the mean number of ampules of human menopausal gonadotropins, peak estradiol levels, the number of oocytes, and the percent of oocytes fertilized were not statistically different. No luteinizing hormone surges were detected with Nal-Glu in serum or urine. Nal-Glu was well tolerated, and five pregnancies have resulted. We conclude that intermittent administration of Nal-Glu is highly effective in achieving down-regulation and blocking spontaneous luteinizing hormone surges. Compared with leuprolide acetate-human menopausal gonadotropins cycles, an equally high oocyte and embryo yield may be anticipated. This new protocol substantially decreases cycle length and increases patient convenience. (AM J OBSTET GYNECOL 1991;165:1806-10.)

Key words: Gonadotropin-releasing hormone agonist, gonadotropin-releasing hormone antagonist, Nal-Glu, in vitro fertilization

The gonadotropin-releasing hormone (GnRH) agonist has been used extensively for down-regulation before ovarian stimulation, to enhance follicle selection and prevent premature luteinization and luteinizing hormone (LH) surges in patients undergoing controlled ovarian hyperstimulation for in vitro fertilization (IVF). Because of the successful experience in IVF with the GnRH agonist, its use has become almost rou-

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Reprint requests: Rogerio A. Lobo, MD, LAC + USC Medical Center, Women's Hospital, Room 1M2, 1240 N. Mission Road, Los Angeles, CA 90033. 6/6/32684 tine.^{2, 3} However, several disadvantages are inherent with its use. An extended treatment period results from the additional 2 to 3 weeks required for down-regulation to occur.⁴ In addition, the initial agonistic effect has led to occasional ovarian cyst formation,^{5, 6} and an increased requirement for gonadotropins^{7, 8} has led to increased costs with its use.

The GnRH antagonist, on the other hand, achieves down regulation rapidly without exerting an agonistic effect and therefore potentially could reduce or eliminate some of these disadvantages of the GnRH agonist. 9. 10 However, histamine allergic-type reactions with several antagonists have precluded their clinical use. Recently, however, [Ac-D-Nal¹, D-4-Cl-Phe², D-Pal³, Arg⁵, D-Glu(AA)⁶, D-Ala¹⁰]GnRH (Nal-Glu) has been used successfully in women without any significant side effects. 11 Frydman et al. 12 have previously demonstrated the effectiveness of Nal-Glu in preventing spon-

taneous LH surges during controlled ovarian hyperstimulation in normal women. We therefore devised a new protocol for using Nal-Glu in IVF to enhance patient acceptance and achieve the same benefits of GnRH agonists while minimizing its disadvantages. We hypothesized that Nal-Glu may need to be administered only intermittently: an initial treatment to achieve down-regulation, then again at midcycle to prevent premature luteinization and LH surges.

Material and methods

Seven women undergoing ovarian hyperstimulation for IVF or gamete donation were studied. The mean age of the patients was 35.1 ± 1.2 years (range 32 to 40 years). All women had previously undergone an IVF cycle with leuprolide acetate and human menopausal gonadotropins. Therefore each woman served as her own control for comparison of the two hyperstimulation protocols.

In the control cycle patients received leuprolide acetate (Lupron, TAP Pharmaceuticals, Deerfield, Ill.), I mg subcutaneously daily beginning in the midluteal phase of the previous menstrual cycle for 14 days or until down-regulation was achieved (estradiol <30 pg/ml). Human menopausal gonadotropins (hMG) (Pergonal, Serono Laboratories, Norwell, Mass.), three to four ampules per day intramuscularly, was begun after down-regulation. Leuprolide acetate was decreased to 500 µg subcutaneously and administered in conjunction with human menopausal gonadotropins until human chorionic gonadotropin (hCG), 10,000 IU, was administered.

In the treatment cycle, patients received the GnRH antagonist Nal-Glu, 50 µg/kg/day intramuscularly, beginning on cycle day 1 or 2 for 3 days or until down regulation was achieved (estradiol <30 pg/ml). Nal-Glu was obtained from the Salk Institute in collaboration with S.S.C. Yen, MD, DSc, University of California, San Diego, La Jolla, Calif. Nal-Glu was then discontinued, and the usual dose of hMG (three to four ampules per day) was administered intramuscularly. When the largest follicles reached 14 to 16 mm, Nal-Glu was restarted and administered daily in conjunction with hMG until hCG was administered.

The protocol for ovarian stimulation and monitoring of each cycle was identical. Transvaginal ultrasonography with a General Electric 3200 was used to monitor follicular growth in conjunction with serum estradiol levels. hCG, 10,000 IU, was administered when the lead follicles reached 20 mm in both leuprolide acetate and Nal-Glu cycles. Patients in the Nal-Glu group collected urine specimens every 4 hours on the day of hCG, before its administration. The specimens were analyzed for the presence of LH with a commercially available enzyme-linked immunoassay (Ovuquick, Monoclonal Antibodies, Mountain View, Calif.). Transvaginally guided follicle aspiration was performed 36 hours after hCG administration. Embryos were replaced transcervically 48 hours after aspiration, into the patient in the case of IVF or into an artificially cycled agonadal recipient for ovum donation. Progesterone supplementation was provided in the form of progesterone in oil, 25 mg intramuscularly, daily in all IVF patients. Donor ovum recipients received hormonal replacement as previously described.18 Serum β-hCG levels were determined 9 and 14 days after embryo transfer. Pregnancy was confirmed by transvaginal ultrasonography.

Blood samples were centrifuged and the sera were extracted and then analyzed for estradiol on the same day by rapid radioimmunoassay (Pantex, Santa Monica, Calif.). Serum samples were then frozen and stored at -20° C until analyzed for LH in a previously validated assay by double-antibody radioimmunoassay.14 Intraassay and interassay coefficients of variation for the estradiol and LH assays did not exceed 7% and 12%, respectively.

Statistical analyses were carried out with the Student t test (paired and unpaired). All values are reported as mean ± SE.

Results

With the use of Nal-Glu, the amount of time required for down-regulation (estradiol, <30 pg/ml) significantly decreased compared with leuprolide acetate-human menopausal gonadotropins cycles (1.6 \pm 0.3, range I to 3 days vs 20.1 ± 4.1 , range 14 to 47 days, p < 0.001) (Fig. 1). Baseline levels of estradiol were significantly higher with leuprolide acetate-human menopausal gonadotropins (120 ± 10 pg/ml) than with Nal-Glu-human menopausal gonadotropins (57.4 ± 10.5 pg/ml, p < 0.05). This difference is reflective of our protocol, where leuprolide acetate is begun in the midluteal phase. Serum estradiol levels after down-regulation with either GnRH agonist or Nal-Glu were comparable.

Serum LH baseline levels were not significantly different in the leuprolide acetate-human menopausal gonadotropins group (11.9 ± 3.4 mIU/ml) compared with the Nal-Glu-human menopausal gonadotropins group (13.7 \pm 1.6 mIU/ml) (Fig. 2). After down-regulation with Nal-Glu, serum LH levels were significantly lower than at baseline (p < 0.01). In addition, serum LH in the Nal-Glu-human menopausal gonadotropins group after down-regulation (6.4 ± 0.9 mIU/ml) was significantly lower than serum LH values of the leuprolide acetate-human menopausal gonadotropins group (10.9 \pm 1.7 mIU/ml, p < 0.05).

In a comparison of serum LH levels in the two groups on the day of hCG administration, in the Nal-Gluhuman menopausal gonadotropins group serum LH was 9.7 ± 0.9 mIU/ml, which was significantly lower than with leuprolide acetate-human menopausal gonadotropins (14.9 \pm 1.5 mIU/ml, p < 0.01). A signif-

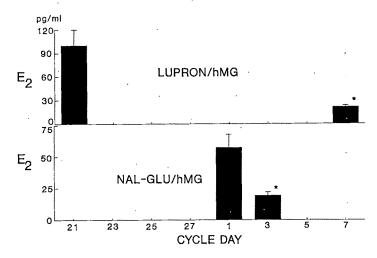


Fig. 1. Comparison of length of time needed for estradiol down-regulation between leuprolide acetate—human menopausal gonadotropins and Nal-Glu—human menopausal gonadotropins regimens. Asterisks, Significant differences between estradiol levels at baseline and at down-regulation (p < 0.05). Estradiol levels at time of down-regulation are not different in two groups.

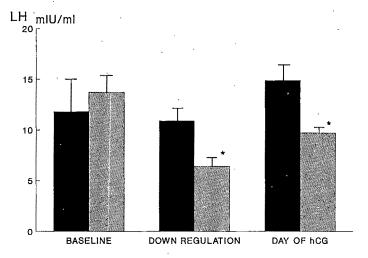


Fig. 2. Comparison of LH levels between leuprolide acetate—human menopausal gonadotropins (solid bars) and Nal-Glu—human menopausal gonadotropins (striped bars) cycles at baseline, after down-regulation, and on day of hCG administration. LH decreased significantly with Nal-Glu—human menopausal gonadotropins (p < 0.05). Asterisks, Differences between two groups at down-regulation (p < 0.05) and on day of hCG (p < 0.01).

icant increase in LH occurred in the leuprolide acetate—human menopausal gonadotropins group during human menopausal gonadotropins stimulation in spite of continued leuprolide acetate administration (serum LH was 10.9 ± 1.7 mIU/ml after down-regulation and 14.9 ± 1.5 mIU/ml on the day of hCG administration, p < 0.05). However, an increase was not observed in Nal-Glu cycles in spite of the fact that Nal-Glu was not administered during this time.

Table I compares the results of treatment in the leuprolide acetate-human menopausal gonadotropins and Nal-Glu-human menopausal gonadotropins groups. The mean cycle length in the Nal-Glu-human menopausal gonadotropins group was significantly shorter than in leuprolide acetate—human menopausal gonadotropins cycles (11.0 \pm 1.0, range 8 to 14 days vs 31.3 \pm 5.8, range 17 to 54 days, p < 0.01). The mean total days on treatment was significantly decreased with the use of intermittent Nal-Glu (6.7 \pm 0.42 vs 31.3 \pm 5.8 days, p < 0.01). The mean number of days of human menopausal gonadotropins treatment (8.3 \pm 0.4 vs 8.7 \pm 0.6), mean number of ampules of human menopausal gonadotropins (29.9 \pm 2.1 vs 31.3 \pm 2.7), peak estradiol levels (2485 \pm 300 vs 2250 \pm 245 pg/ml), number of oocytes obtained (13.9 \pm 3.0 vs 11.9 \pm 1.2), and percent of oocytes fer-

Table I. Comparisons between two treatment protocols

	Leuprolide acetate-hMG	Nal-Glu-hMG	p Value
Time to down-regulation (days)	20.1 ± 4.1	1.6 ± 0.3	< 0.001
Total length of agonist-antagonist treatment (days)	31.3 ± 5.8	6.7 ± 0.42	< 0.01
hMG (days)	8.3 ± 0.4	8.7 ± 0.6	NS
Cycle length (days)	31.3 ± 5.8	11.0 ± 1.0	< 0.01
Ampules of hMG	29.9 ± 2.1	31.3 ± 2.7	NS
Peak estradiol (pg/ml)	2485 ± 300	2250 ± 245	NS
Oocytes (No.)	13.9 ± 3.0	11.9 ± 1.2	NS
Fertilization (%)	53.9 ± 11.6	60.3 ± 10.2	NS

NS, Not significant.

tilized (53.9% \pm 11.6% vs 60.3% \pm 10.2%) were not significantly different between the two groups. Three pregnancies have resulted from treatment in the leuprolide acetate-human menopausal gonadotropins group, and five pregnancies have occurred in the Nal-Glu-human menopausal gonadotropins group.

Nal-Glu was well tolerated. No complaints of hypoestrogenism (i.e., hot flushes) were observed. Two women developed a histamine reaction resulting in a pruritic allergic wheal. These symptoms resolved within 1 day after cessation of Nal-Glu.

Comment

The administration of a GnRH agonist daily initially stimulates and then suppresses pituitary-ovarian function through a desensitization of the pituitary.15 The total time required for down-regulation has been shown to be approximately 14 days.4 With the administration of Nal-Glu, the time required to achieve downregulation is significantly reduced. Acting as a competitive inhibitor of gonadotropin-releasing hormone, Nal-Glu lacks an agonistic effect and directly blocks the pituitary, preventing hypothalamic GnRH-induced gonadotropin release.16 When Nal-Glu is used as an adjunct for stimulation with gonadotropins, down-regulation is achieved more readily and the total cycle length is decreased because the agonistic phase is eliminated. This substantially increases patient convenience.

The administration of Nal-Glu resulted in significantly lower levels of LH after down-regulation, in comparison with treatment with the agonist leuprolide acetate. The rationale behind the down-regulation of patients with Nal-Glu was to better compare these cycles with our established hyperstimulation protocol with leuprolide acetate-human menopausal gonadotropins. Serum LH levels declined within 24 hours of Nal-Glu administration, whereas no significant decrease in LH levels was noted during down-regulation with leuprolide acetate. This illustrates the more effective inhibition of gonadotropin release by the antagonist versus the agonist. Our data therefore support the notion that Nal-Glu provides a short, convenient regimen for use in IVF, accomplishing acute and rapid pituitary down regulation, as well as preventing premature luteinization and LH surges. In addition, the need for continuous hypothalamic-pituitary suppression appears not to be essential as demonstrated by our protocol. Intermittent administration of the GnRH antagonist (initially and at midcycle) may be all that is necessary for prevention of premature luteinization and LH surges.

On the day of hCG administration, significant suppression of LH by Nal-Glu was evident when compared with the cycles where leuprolide acetate was administered. Patients treated with leuprolide acetate showed a significant increase in serum LH levels as a result of ovarian hyperstimulation, in spite of continuous leuprolide acetate administration. However, the midcycle LH surge was inhibited. Whether this small increment in LH, which occurred with stimulation in leuprolide acetate cycles, is of significance in terms of follicular luteinization and oocyte quality is unknown at present. Although the wide success achieved with leuprolide acetate in IVF cycles speaks for itself, elevated LH levels have been implicated in failures in IVF because of poor oocyte quality and in early abortion.17, 18

In this preliminary study we were unable to find a difference between the two protocols in the number of days of human menopausal gonadotropins treatment and in the number of ampules required for ovarian stimulation. Previous studies7,8 have shown an increase in the number of ampules required when down-regulation with GnRH agonist is used. This has been suggested to be due to either the suppressed levels of endogenous bioactive LH and follicle-stimulating hormone by the GnRH agonist, resulting in an increased need of exogenous stimulation for follicle recruitment, or a direct inhibiting effect of the agonist on the ovary. Our data showed no differences in the requirement for gonadotropins with leuprolide acetate or Nal-Glu. While it is possible that Nal-Glu may have resulted in a decreased requirement of gonadotropins with larger patient groups, our data are more supportive of the first notion, that profound inhibition of endogenous gonadotropins results in a greater requirement of exogenous gonadotropins.

Pregnancies resulted from both treatment cycles. In the leuprolide acetate—human menopausal gonadotropins cycles, three pregnancies occurred (two from IVF and one from donor oocytes after treatment with leuprolide acetate—human menopausal gonadotropins). However, no pregnancy is currently in progress. In the Nal-Glu—human menopausal gonadotropins cycles, five pregnancies resulted (two from IVF and three from donor oocytes after Nal-Glu—human menopausal gonadotropins). Of these, the three recipients of donor oocytes are currently pregnant. Although our numbers are too small to demonstrate a difference, or lack of one, in terms of pregnancy rates with the two treatments, Nal-Glu cycles were clearly able to provide healthy oocytes that could result in pregnancy.

The ease and efficiency of the use of the GnRH antagonist Nal-Glu for down-regulation and in preventing the midcycle LH surge during ovarian hyperstimulation for IVF has been demonstrated. Although our study population is small, this preliminary study suggests that Nal-Glu does not produce any detrimental effects and is as effective as other currently used hyperstimulation regimens.

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The gonadotropin-releasing hormone antagonist (Nal-Glu) acutely blocks the luteinizing hormone surge but allows for resumption of folliculogenesis in normal women

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The gonadotropin-releasing hormone antagonist offers several advantages over the use of the agonist and allows several physiologic questions to be addressed. In this study, we evaluated the ability of Nal-Glu to acutely inhibit the luteinizing hormone surge and prevent ovulation. We also assessed whether recovery of the follicle would be possible after several days of gonadotropin deprivation and estradiol decrement. Eight normal ovulatory women were randomized to control or Nal-Glu-treated cycles (50 µg/kg intramuscularly) for 3 to 4 days. Monitoring was carried out with daily vaginal ultrasonographic scans and serum estradiol levels and twice-daily serum luteinizing and follicle-stimulating hormone levels. Nal-Glu acutely inhibited the luteinizing hormone surge and ovulation, even when administered as late as the onset of the luteinizing hormone surge. Evidence was provided that spontaneous follicular rescue recurred in eight of 10 cycles after 3 to 4 days of Nal-Glu administration. Although an estradiol to follicular size dissociation occurred with Nal-Glu, subsequent ovulation occurred in 5.1 ± 0.6 days after the last Nal-Glu dose. The decrement in estradiol after Nal-Glu administration correlated negatively with the days required for subsequent ovulation to occur (r = 0.77, p < 0.05). The subsequent luteal phase also was normal in terms of length and progesterone levels. These data confirm the potency and efficacy of Nal-Glu in acutely inhibiting gonadotropins and extends our knowledge on the physiologic characteristics of the dominant follicle. (Am J OBSTET GYNECOL 1991;165:1811-7.)

Key words: Nal-Glu, gonadotropin-releasing hormone antagonist, follicle growth, ovulation inhibition

Use of the gonadotropin-releasing hormone (GnRH) agonist has become commonplace in the treatment of several reproductive disorders and has been useful as an adjunctive treatment for ovarian stimulation with gonadotropins. In ovarian stimulation for in vitro fertilization, its use has been helpful in preventing premature luteinization and premature luteinizing hormone (LH) surges. By virtue of ovarian suppression before gonadotropin stimulation, use of GnRH agonist has resulted in a greater number of oocytes obtained when used in cycles in in vitro fertilization.1,2

With the GnRH agonist, however, the goal of down regulation may require 1 to 3 weeks to achieve.3 It is also associated with an initial agonistic (stimulatory) phase that may lead to ovarian cyst formation. With the use of GnRH antagonists, however, these disad-

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pin levels and does not exhibit an agonistic effect.4 However, because of relatively low potency and the induction of histamine release by mast cells, the clinical application of GnRH antagonists has been limited.5 Nal-Glu [Ac-D-Nal¹, D-4-Cl-Phe², D-Pal³, Arg⁵, D-Glu (AA)⁶, D-Ala10], a GnRH antagonist that is several times more potent than those previously reported and has low mast cell secretagogue activity,6 has been developed7 and tested clinically.8-16 Nal-Glu is a decapeptide similar to GnRH except for substitutions of amino acids at positions 1, 2, 3, 4, 5, 6, and 10. A naphthylalanine residue is at position 1, a 4-Cl-phenylalanine at position 2, phenylalanine-ammonia-lyase at position 3, arginine residue at position 5, D-4-(p-methoxybenzoyl)-2 aminobutyric acid at position 6 and alanine at position 10. The ability of Nal-Glu to acutely inhibit gonadotropin secretion has allowed several physiologic questions to be answered.

vantages may be overcome, because the antagonist in-

duces an immediate decrease in circulating gonadotro-

Our purpose in this study was to determine whether the antagonist Nal-Glu is able to acutely inhibit the LH surge and prevent ovulation when administered only at midcycle to normal women. Furthermore, we sought to determine if follicle rescue may occur with gonad-

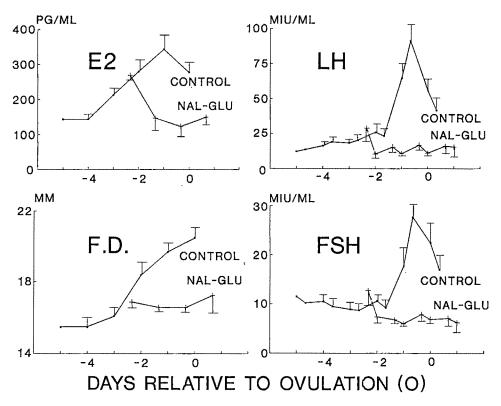


Fig. 1. Mean \pm SE of estradiol (E2), LH, FSH, and follicular diameters (F.D.) at midcycle in eight ovulatory women monitored during control cycles, compared with their data in Nal-Glu cycles.

otropin recovery after several days of gonadotropin deprivation and estradiol decrement. If Nal-Glu were to be successful in acutely inhibiting the spontaneous LH surge, it would prove to be valuable as an adjunct for induction of ovulation with menotropins.

Material and methods

Subjects. Eight normal, cycling women aged 22 to 35 years (31.4 ± 6) were studied. All women were ovulatory with documented cycle lengths of 25 to 31 days and were using a mechanical form of contraception at the time of enrollment. The subjects were in good health and received no medications during the study period. No woman had received any hormonal therapy for at least 2 months before the study. The project was approved by our institutional review board, and written informed consent was obtained from each subject.

Protocol. All eight women were studied during two consecutive cycles consisting of a control and a treatment cycle, the sequence of which was randomly determined. The control cycle consisted of daily transvaginal ultrasonography performed by the same individual, beginning once a patient's lead follicle had reached 16 mm. Follicular diameters were taken to be the maximum diameter of several measurements carried out for each follicle. Ultrasonographic scanning continued daily until each follicle was seen to rupture. The Siemens Sonoline SLI model with a 5.0 MHz vag-

inal probe was used for ultrasonographic monitoring. Blood samples were obtained twice daily, at 8 AM and 4 PM, once maximum follicular diameters reached 16 mm. Seven days after ovulation, as documented by ultrasonography, blood was drawn at 8 AM for progesterone levels.

In the treatment cycles monitoring was carried out in an identical fashion. Once follicle sizes reached 16 to 18 mm, Nal-Glu (50 μ g/kg) was administered intramuscularly each morning at 8 AM. Nal-Glu was obtained from the Salk Institute in a collaborative project with S.S.C. Yen, MD, DSc, University of California, San Diego. The 50 μ g/kg dose was chosen on the basis of previously reported dose-response studies in which a maximal suppression of LH (51% to 63%) occurred in postmenopausal women within 5 to 8 hours; the duration of this effect was 24 hours.¹⁷ Nal-Glu was administered for 3 to 4 consecutive days, the time estimated to prevent normal ovulation on the basis of known patterns of follicle growth.

Hormonal assays. All blood was separated, and sera were stored at -20° C until analyzed. Serum LH, follicle-stimulating hormone (FSH), estradiol, and progesterone were measured by highly specific radioimmunoassays. ^{18, 19} Serum estradiol concentrations were measured by radioimmunoassay (Pantex, Los Angeles) after extraction with hexane/ethyl acetate (2:3). In addition, LH also was measured by time-resolved fluo-

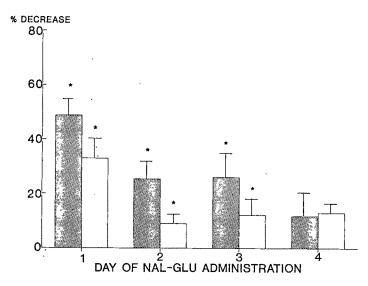


Fig. 2. Mean ± SE percentage decrease of LH (hatched bars) and FSH (dotted bars) between 8 AM and 4 РМ during Nal-Glu (50 μ g/kg intramuscularly) administration at 8 Λ M. Asterisks, Decreases (p < 0.01) in LH and FSH as compared with baseline levels.

roimmunoassays at SmithKline Beecham Clinical Laboratories, Van Nuys, Calif., with Delfia human LH spec kits, according to the method of Lovgren et al.20 All sera from each patient were run in a single assay to decrease variation. The mean intraassay and interassay coefficients of variation for the serum radioimmunoassays were as follows: LH, 2.7% and 10%; FSH, 5% and 10%; estradiol, 8.4% and 13%; and progesterone, 6.2% and 7%, respectively. The intraassay and interassay coefficients of variation for the LH serum fluoroimmunoassays were 5% and 7%, respectively.

Statistical analyses. All data are expressed as the mean ± SEM. Statistical analyses were performed with paired t tests, and regression analyses were carried out with Pearson's regression analysis.

Results

Midcycle data of the eight normal ovulatory women during control cycles, compared with their data in Nal-Glu cycles, are depicted in Fig. 1. With Nal-Glu, gonadotropins were abruptly inhibited, and ovulation did not occur according to ultrasonographic criteria. Nal-Glu also decreased serum estradiol levels and arrested follicle growth. Serum estradiol decreased by 43.1% = 8%, $53.2\% \pm 9\%$, $50\% \pm 15\%$, and 47% ± 13% in comparison with pretreatment baseline values during the 4 days of Nal-Glu administration. The follicular diameters remained essentially unchanged (16.9 \pm 0.4 to 17.25 \pm 1.1 mm). When Nal-Glu was matched to control cycles, the initial day of Nal-Glu administration corresponded to 2.2 days before ovulation.

Serum LH values at 8 AM decreased significantly during Nal-Glu treatment. Mean values were 29 ± 9, 16 ± 2 , 17 ± 2 , and 16 ± 7 mIU/ml during Nal-Glu

treatment. Corresponding values for FSH were 13 ± 3, 7 ± 1 , 8 ± 1 , and 7 ± 1 mIU/ml. The percentage changes in LH and FSH after each dose of Nal-Glu is depicted in Fig. 2. These percentages represent the changes between the 8 AM and 4 PM blood samples. Serum LH levels consistently decreased more than FSH levels except on day 4, when these changes were similar.

We also measured LH by fluoroimmunoassay. LH levels decreased by $71.3\% \pm 6.5\%$, $61.3\% \pm 6.3\%$, $48.8\% \pm 7\%$, and $26.1\% \pm 9\%$. This percentage decrease was significantly greater than the radioimmunoassay measurements on days 2 to 4 (p < 0.05).

In treatment cycles, after the last dose of Nal-Glu, spontaneous follicular rescue occurred in eight of 10 cycles. Fig. 3 illustrates the LH, FSH, and estradiol levels and follicular diameters during the days from the last Nal-Glu injection until the subsequent ovulation. On the average, 4.1 ± 0.6 days (range 1 to 8) was required for the subsequent gonadotropin surge to occur and 5.1 ± 0.6 days (range 3 to 9) for the patients to ovulate, as assessed by ultrasonography. The average follicular diameter of 16.9 ± 0.5 mm on the last day of Nal-Glu increased to a maximum of 19.3 ± 0.7 mm, whereas the average estradiol measurement increased from 128.5 ± 20.7 pg/ml on the last day of Nal-Glu to $337 \pm 20.8 \text{ pg/ml} (p < 0.01).$

Table I summarizes the effect of Nal-Glu at midcycle by using each patient's control cycle as reference. Of the eight patients receiving Nal-Glu, two had repeat cycles, resulting in 10 monitored cycles. The individual patients are listed with their observed normal cycle lengths, their estradiol and follicular diameter values at the beginning of Nal-Glu administration, the cycle days on which Nal-Glu was given, and when ovulation occurred after Nal-Glu administration. Patient 2 un-

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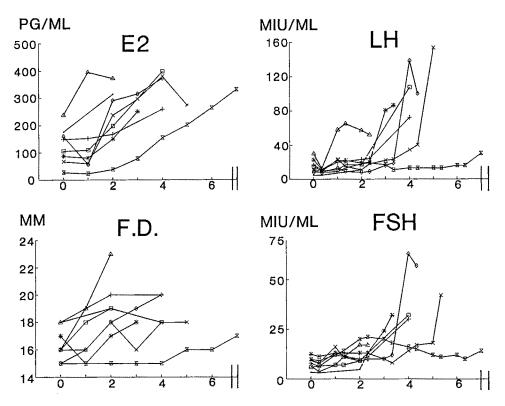


Fig. 3. Individual patient profiles of estradiol (E2), LH, FSH, and follicular diameters (F.D.) during days from last Nal-Glu injection until subsequent ovulation.

Table I. Effect of Nal-Glu at midcycle with each patient's control cycle used as reference

Patient No.	Cycle length (days)	Estradiol/follicle diameter (start)	Nal-Glu (days)	Ovulation day (after Nal-Glu)
1	26	268/18	11-14	19
2*	29	243/18	11-14	NA
3†	24	257/18	13-16	20
4	29	274/18	12-14	18
5	29	383/17	12-14	19
6	27	213/16	11-14	20
7	30	277/18	12-14	NA
8	27	325/16	12-14	19
2	29	238/18	12-14	17
3	24	211/14	12-14	23

NA, Not available.

derwent a second Nal-Glu injection because a secondary follicle developed although the LH surge was inhibited. This patient probably had luteinization of two follicles in that they persisted and did not continue to grow. The progesterone level was 4.5 ng/ml 4 days later. Patient 3 also had a repeat cycle because she had an LH surge (105 IU/ml) the day she was first seen, before receiving the first dose of Nal-Glu. However, in this cycle ovulation did not occur and there was subsequent recovery. In a subsequent cycle Nal-Glu was administered 1 day earlier, although the follicle size was

only 14 mm. With patient 7 the cycle was interrupted irreversibly and there was no recovery. She bled 2 days after Nal-Glu when the estradiol level had decreased to 67 pg/ml from a value of 277 pg/ml on the first day of Nal-Glu. Subsequently, this patient had a normal cycle, and a new follicle was observed to develop.

We compared the estradiol/follicular diameter ratio in control cycles before ovulation (13.6 \pm 1) and during the last day of Nal-Glu (7.5 \pm 1.2) and immediately before the subsequent ovulation after recovery from Nal-Glu (16.8 \pm 1.1). The mean estradiol/follicular di-

^{*}Double-dominant (luteinized follicles).

[†]Surge before Nal-Glu.

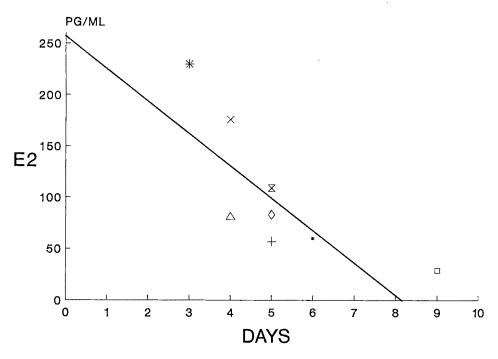


Fig. 4. Correlation between lowest estradiol (E2), level during Nal-Glu administration and time required for subsequent ovulation to occur (r = 0.77, p < 0.05).

ameter ratio in the control group was significantly different from the other two treatment times (p < 0.01and p < 0.02).

A significant negative correlation was found between the lowest estradiol value after Nal-Glu and the number of days required for subsequent ovulation (r = -0.77, p < 0.05 (Fig. 4).

Luteal function after recovery from Nal-Glu was assessed by comparing the length of the subsequent luteal phase and midluteal progesterone levels with control cycles. Luteal length was 14 ± 0.3 days (range 13 to 15) in control cycles and 14.4 ± 1.0 days (range 12 to 19) after documented ovulation in Nal-Glu cycles. Serum progesterone values 7 days after documented ovulation were similar in the two groups and were 15.5 ± 2.3 ng/ml (range 10 to 16) and 18.8 ± 2.1 ng/ml (range 11 to 26), respectively.

Nal-Glu was well tolerated. A slight discomfort for about 20 to 30 minutes was noted after the first injection. No patient had aberrant responses.

Comment

Our data show that Nal-Glu is able to acutely prevent ovulation when administered at midcycle. Although the GnRH antagonist has been used in the follicular phase in previous studies, our data suggest that when it is administered as late as the day of the LH surge (patient 3), ovulation was also inhibited. In addition, evidence was provided that follicular rescue occurs after Nal-Glu with subsequent ovulation occurring in eight of 10 cycles. Recovery after Nal-Glu administration was negatively correlated with the decrement in estradiol with treatment. With subsequent ovulation, apparent normal luteal function was found to occur, although this assessment was based only on a single serum progesterone measurement. Whereas the viability of the oocyte after several days of arrested follicular growth remains uncertain, pregnancies have resulted after in vitro fertilization when Nal-Glu was used.21 During Nal-Glu treatment and subsequent recovery, an estradiol/follicular diameter dissociation was evident, as compared with control cycles.

At midcycle, LH was inhibited by 12% to 49% by radioimmunoassay and 36% to 71% by fluoroimmunoassay. The fluoroimmunoassay measurement is thought to better reflect the biologic activity of LH and is compatible with the known effects of the antagonist on LH.22 The greater inhibition of LH compared with FSH has been noted by other investigators.^{23, 24} These differences could be explained either by differences in half-lives of the gonadotropins or by a differential regulation of secretion.

Correlations were carried out for changes in the gonadotropins, estradiol, and follicular diameters in the control cycles, whereas patients were receiving Nal-Glu and after Nal-Glu during the recovery period. Serum LH always correlated significantly with FSH (p < 0.01). Serum estradiol correlated significantly with follicular diameters (p < 0.01), except during Nal-Glu administration.

Our recovery data suggest that even after sustained gonadotropin deprivation at midcycle, follicular recovery is possible in at least eight of 10 cycles. That the time to recovery was inversely correlated with the suppressed estradiol levels suggests that profound or more prolonged suppression may result in follicle atresia. With recovery, estradiol levels rapidly increased, whereas follicle size did not increase commensurately. This observation suggests that follicle growth and its steroidogenesis may be dissociated and may not be under the same regulatory control.

Although it was determined in monkeys >10 years ago that destruction of the dominant follicle resulted in a subsequent follicle development requiring 10 to 12 days,25,26 a variable recovery of the dominant follicle was noted to occur after another GnRH antagonist was used in monkeys at midcycle.27 Furthermore, Detirelix, another second-generation antagonist, which is less potent than Nal-Glu,17 was reported recently to inhibit follicular development in the late follicular phase, allowing for subsequent recovery and ovulation in four normal women.28 That recovery of a dominant follicle would occur after 3 to 4 days of antagonist administration and 5.4 ± 1.4 days of estradiol decrement was somewhat of a surprise but did occur in 80% (8/10) of our cycles tested. However, it appears obvious that more prolonged suppression of estradiol may lead to irreversible follicular or oocyte atresia.

Nal-Glu appears to be useful in in vitro fertilization for down regulation before gonadotropin stimulation and for preventing premature luteinization and a premature LH surge. Our data would suggest that for preventing a premature LH surge Nal-Glu may be administered extremely late in folliculogenesis and need be administered only just before the LH surge. If it is administered earlier in the follicular phase, supplementation with gonadotropin would be necessary to prevent follicles from becoming atretic.

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Reduction of primary posttraumatic adhesion formation with the prostacyclin analog iloprost in a rodent model

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Recent evidence suggests that inhibition of postsurgical adhesion formation may be effected by modulation of the activities of inflammatory cells contributing to mesothelial repair. Iloprost, a stable analog of prostacyclin, has been shown to exert vasodilatory, antiinflammatory, fibrinolytic, and antithrombotic influences. To determine whether these properties of iloprost might protect mesothelial surfaces from perioperative damage and hence prevent adhesion formation, we evaluated the effect of iloprost on peritoneal healing in a hamster model for primary pelvic injury. Perioperative iloprost therapy significantly reduced posttraumatic adhesion formation when compared with that in vehicle-treated controls. Doseresponse studies demonstrate adhesion prevention with doses ranging from 0.04 to 4 mg/kg per 8 hours given subcutaneously over the course of 3 days. These data demonstrate that iloprost is a potent positive modulator of peritoneal healing after pelvic trauma. Further studies to characterize the potential application of iloprost as an adjuvant in reproductive surgery are indicated. (AM J OBSTET GYNECOL 1991;165:1817-20).

Key words: Iloprost, adhesion prevention, infertility surgery

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Postsurgical adhesion formation is a nonspecific inflammatory process directed primarily by activated peritoneal macrophages. Contemporary research in the development of adhesion prevention regimens emphasizes the use of immunomodulation of inflammatory cell activity to reduce mesothelial injury and to enhance normal reperitonealization. Iloprost, a stable analog of prostacyclin, has been shown to exert vasodilatory, antiinflammatory, fibrinolytic, and antithrombotic influ-

Table I. Scoring system for postsurgical adhesion formation

Adhesion score	Location	Thickness	Extent
0 1+ 2+	No adhesions Intrauterine horn adhesions Adhesions from the uterine horn to bowel	No adhesions Filmy adhesions Thick adhesions	No adhesions Adhesions covering <50% of injured surface Adhesions covering >50% of injured surface

ences. We hypothesized that these properties might be used to influence posttraumatic mesothelial repair and to reduce the incidence of intraperitoneal adhesion formation. We present the results of preliminary experiments evaluating the effect of iloprost on primary posttraumatic healing in a rodent model.

Methods

The hamster model for primary posttraumatic peritoneal repair used in these experiments was derived from procedures previously developed and validated in our laboratory. Sexually mature female golden hamsters (Harlan-Sprague Dawley, Indianapolis) that were 6 to 8 weeks of age and that weighed 100 to 150 gm) were observed in quarantine for 10 days before use in these experiments. They were fed standard laboratory chow and water as desired and were maintained on a cycle of 14 hours light/10 hours dark.

A standardized adhesiongenic lesion was performed on each animal at laparotomy. Preoperatively each animal received 1.0 mg of penicillin G (Squibb, Princeton, N.J.) subcutaneously 30 minutes before surgery. Anesthesia was induced by intraoperitoneal injection of pentobarbital, 0.5 mg (Abbott Laboratories, North Chicago). Midline laparotomies were performed under clean but not sterile conditions. Care was taken to maintain gross hemostasis during the procedure. At laparotomy the left uterine horn was identified and brought out of the wound. Battery-powered unipolar ophthalmologic cautery was used to create a standardized adhesiongenic lesion consisting of devascularization of the entire vascular arcade of the left uterine horn, placement of 12 to 15 serosal burns along the distal 3 cm of the antimesenteric surface of the horn, and the opening of a 1.5×1.5 cm defect in the mesentery. This lesion had previously been shown to produce adhesions between the uterine horn and the bowel and/or pelvic sidewall in >90% of treated animals. The right uterine horn was not manipulated and served as an internal control. Abdominal closures were performed in two layers with 4-0 polyglycolic acid sutures.

Immediately before surgery, hamsters were randomly assigned to control or experimental treatment groups. Each animal received nine subcutaneous injections of the study medications at 8-hour intervals that began 30 minutes before operation. Experimental treatments included: vehicle (saline solution, n = 10)

and iloprost (Berlex Laboratories, Cedar Knolls, N.J.) in doses of 4, 0.4, 0.04, and 0.004 mg/kg (n = 10 per group). Dosage intervals were based on the amount of iloprost used to modulate cardiovascular function in rodents.²

Two weeks after the initial laparotomy, all animals were killed by cervical dislocation for assessment of adhesion formation. The location, extent, and thickness of induced adhesions were separately graded in a blinded manner by the original surgeons on a scale ranging from 0 to 2+ (Table I), which yielded a maximum cumulative score of 6+. All data are reported as the mean \pm SEM. Nonparametric statistics (Kruskal-Wallis and Mann-Whitney U tests) were used to compare the outcome of postsurgical healing.

Results

The standardized adhesiongenic lesion used in this model, in accordance with our previous experience, consistently induced the formation of dense adhesions between the injured uterine horn and the bowel and pelvic sidewall. The mean adhesion score for control animals was 5.8 ± 0.2 . Nine of 10 hamsters were found to have maximal (6+) adhesive involvement at necropsy. No control hamster scored <4+.

Administration of nine perioperative doses of iloprost produced a dose-dependent reduction in adhesion formation (Table II). Maximal adhesion reduction was seen in hamsters receiving the 4 mg/kg dose. Mean total adhesion score in this group was 0.7 ± 0.5 (different from control, p < 0.001, Kruskal-Wallis test). Eight of 10 animals in this group were completely free of adhesions at necropsy. Significant adhesion reduction also was observed with the doses of 0.4 and 0.04 mg/kg (total score, 1.3 ± 0.5 and 1.9 ± 0.5 , respectively). Reductions in total adhesion scores in these three groups were the result of an equal decrement in the scores for the separate parameters of adhesion location, thickness, and extent. Hamsters treated with a dose of 0.004 mg/kg were found to have extensive adhesion formation (average score, 5.2 ± 0.3), which was not different from controls.

Perioperative treatment with iloprost was not associated with significant postsurgical morbidity. No evidence of increased intraperitoneal infection, failure of wound healing, or obvious bleeding dyscrasias was observed. Hamsters treated with the highest dose of ilo-

Table II. Effect of iloprost on primary posttraumatic adhesion formation

	Location	Thickness	Extent	Total score
Control	1.9 ± 0.1	2	1.9 ± 0.1	5.8 ± 0.2
Iloprost, 4 mg/kg	$0.2 \pm 0.1*$	$0.3 \pm 0.2*$	$0.2 \pm 0.1*$	$0.7 \pm 0.5*$
Iloprost, 0.4 mg/kg	$0.4 \pm 0.2*$	$0.5 \pm 0.2*$	$0.4 \pm 0.2*$	$1.3 \pm 0.5*$
Iloprost, 0.04 mg/kg	$0.6 \pm 0.2*$	$0.6 \pm 0.2*$	$0.6 \pm 0.1*$	$1.9 \pm 0.5*$
Iloprost, 0.004 mg/kg	1.5 ± 0.2	2	1.7 ± 0.2	5.2 ± 0.3

^{*}Differs from control (p < 0.001, Kruskal-Wallis test).

prost (4 mg/kg) were noted to display increased agitation when compared with control animals.

Comment

Peritoneal repair after traumatic or infectious injury is essentially a nonspecific inflammatory process, the course of which is dependent on the interaction of numerous immune mediators and inflammatory cells. Studies by Raftery^s and others have divided this process into three stages: an acute postinjury phase, initiation of fibrinolysis and clot resorption 48 hours later, and reperitonealization over the ensuing 14 days. The activated peritoneal macrophage appears to be the principal director of cellular elements that contribute to mesothelial repair.4 Other inflammatory cells, including neutrophils, endothelial cells, platelets, and fibroblasts, may contribute to posttraumatic healing. Under ideal conditions, macrophage-directed reperitonealization produces complete restoration of mesothelial continuity without evidence of scarring or previous trauma. In contrast, excessive tissue injury or exposure to inflammatory stimuli may lead to the formation of dense sheets of adhesions that connect adjacent structures and interfere with normal organ function.

Researchers studying problems in intraperitoneal healing and adhesion prevention have long recognized that the course of peritoneal wound repair may be favorably altered by modifying the activities of inflammatory cells to reduce nonspecific tissue injury. Early investigators viewed this phenomenon largely as an "allor-none" phenomenon. Their goal was to entirely eliminate the phagocytic component of the acute postinjury phase by obliterating the inflammatory response to tissue trauma. Powerful immunosuppressive agents such as corticosteroids were used for this purpose.5 Other important aspects of peritoneal repair were largely ignored. More recent data have shown that immunocompetent cells have an essential role in reperitonealization that extends beyond the acute postinjury phase. It has become increasingly apparent that the goal of contemporary adhesion prevention regimens should be to redirect the activity of inflammatory cells to obtain posttraumatic tissue homeostasis (e.g., prevention of infection, phagocytosis of injured cells) without comprising the essential activity of these cells in the subsequent

phases of fibrinolysis and macrophage-directed reperitonealization. Thus investigators have more carefully examined the pathophysiologic factors of peritoneal repair to elucidate the specific contributions of various inflammatory cells to peritoneal healing and adhesion formation.

Platelet activation, platelet-endothelial cell interaction, and thrombosis are commonly overlooked, but they are essential contributors to each phase of posttraumatic peritoneal repair. Platelet activation after tissue disruption results in the release of a variety of inflammatory mediators and growth factors (platelet-derived growth factor, thromboxane A2, platelet factor 4, transforming growth factor-β, connective tissue activating peptide III, β-thromboglobulin, and epidermal growth factor), which attract and activate inflammatory cells. Platelet activation and release of platelet-derived mediators may adversely affect the sequential aspects of peritoneal healing by increasing nonspecific phagocytic damage and by stimulating fibroblast proliferation and collagen deposition. This process may lead to cellularization of fibrinous adhesions that bind adjacent intraperitoneal structures and to aberrant collagen deposition and the formation of dense cellular intraperitoneal adhesions.

Prostacyclin, a product of the cyclooxygenase pathway of prostaglandin synthesis, is an essential regulator of vascular tone, platelet function, and thrombosis after vascular injury. Review of the prostacyclin literature suggests that this eicosanoid may exert direct and indirect influences on the inflammatory milieu of injured tissue. Vascular elaboration of prostacyclin may reduce nonspecific phagocytic tissue injury by inhibiting the production of inflammatory cell chemotactic and activating factors and by ameliorating inflammatory cell activation (inhibition of neutrophil superoxide6 and leukotriene B4 production,7 inhibition of lymphocyte proliferation,8 and inhibition of macrophage Ia expression9). Fibrinolysis, a critical step in mesothelial healing, is moderately stimulated by prostacyclin.

As a result of knowledge of the effect of prostacyclin on platelet activation, thrombotic homeostasis, inflammatory cell activation, and fibrinolysis, we theorized that prostacyclin analogs might be useful as modulators of posttraumatic peritoneal healing to reduce perioperative adhesion formation. Iloprost, a long-lasting orally active prostacyclin analog, shares many properties of the parent compound. Vasodilatory, platelet-suppressant, fibrinolytic, and "cytoprotective" activities of iloprost equal or exceed those of prostacyclin.10, 11 The results of this preliminary investigation demonstrate that perioperatively administered iloprost is a potent inhibitor of primary pelvic adhesion formation. This activity occurred in a dose-dependent manner; maximal adhesion reduction was observed with a dose of 4 mg/kg. The therapeutic index of iloprost in this indication was fairly broad; significant adhesion prevention occurred over two orders of magnitude. Reduction of total adhesion score was the result of equal contributions from adhesion location, thickness, and extent. Perioperative administration of iloprost did not lead to increased infectious morbidity, failure of wound healing, or obvious bleeding disorders.

These data provide additional support to the emerging concept of the use of "immunomodulatory" (as opposed to "antiinflammatory") agents to enhance postsurgical peritoneal repair. In recent communications we have reported the results of studies in which calcium channel blocking agents, pentoxifylline, and bioactive peptides such as somatostatin and synthetic substance P antagonists (unpublished observations) have been used to prevent posttraumatic adhesion formation in animal models. These agents are not considered to be classic antiinflammatory agents. Rather, these distinctly different classes of agents appear to affect peritoneal repair by modulating the activities of inflammatory cells participating in the adhesion formation cascade. The beneficial influence of the previously mentioned immunomodulatory drugs on adhesion formation appears to result primarily from alterations of macrophage and neutrophil function. Modulation of platelet function with iloprost may provide another means by which to perturb the adhesion formation cascade. Further studies designed to examine the pharmacokinetics and mechanism of iloprost-mediated adhesion prevention and its potential application in the clinical setting are indicated.

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The effect of the menstrual cycle and of decompression stress on arachidonic acid—induced platelet aggregation and on intrinsic platelet thromboxane production in women compared with men

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Menstrual cycle variations in platelet aggregation and thromboxane production in association with sex steroids have been reported. External stimuli such as decompression sickness have been associated with clotting activity changes, specifically, increased platelet aggregation. Differences in response of platelets from women and men, when subjected to such a stress, have been observed. This study evaluated the ability of washed platelets from women in the proliferative and secretory phases of the menstrual cycle to aggregate in response to arachidonic acid and the aggregation difference between washed platelets from women and men in response to decompression stress and arachidonic acid. Additionally, platelet thromboxane production differences between the assessed platelet populations were compared. Our results indicate no difference in platelet aggregability between phases of the menstrual cycle. A significant aggregation difference between platelets from women and men was noted. Platelets from women were more sensitive to arachidonic acid aggregation. These differences were not affected by decompression stress. No difference in thromboxane B2 production was noted between the platelet populations evaluated. (AM J OBSTET GYNECOL 1991;165:1821-9.)

Key words: Platelet aggregation, sex differences, decompression stress response

The relationship of arachidonic acid and its metabolites to platelet aggregation has been a source of intense interest to the hematologist and the endocrinologist. The physiologic actions of arachidonate metabolites and of platelet activities have been of interest to the obstetrician-gynecologist only recently. In 1985 Ylikorkala and Makila¹ published an extensive report reviewing a number of relationships between arachidonic acid metabolite activity and reproductive tract malfunction. Their article focused on thromboxane and prostacyclin and their effect on menorrhagia, uterine contractility, endometriosis, preeclampsia, and gynecologic cancer. Other studies have focused on changes in platelet aggregation during the menstrual cycle² and in relationship to sex steroids.^{3,4} Additional studies have addressed platelets as a source of sex steroids3 and the role of platelet antiglobulins in the pathogenesis of preeclampsia. These studies leave unanswered the true effect of sex steroids on platelet aggregation and the

possibility that aggregation might vary between sexes. If such a difference were true, then women might be at risk for vascular thrombosis during the menstrual cycle and at greater risk than men in some work environments.

One such work area is the aerospace industry with increasing roles of women as pilots and, more recently, as astronauts. Sex differences in decompression sickness and other altitude pressure—related phenomena have appropriately attracted attention. ⁷⁻⁹ Decompression sickness has been associated with altered clotting activity, specifically, increased platelet aggregation. ¹⁰⁻¹² The possibility that sex differences may exist between platelets from women and men and their ability to aggregate when subjected to chemical and physical stimuli also has prompted considerable interest. ¹³⁻¹⁸ These data also leave unclear whether intrinsic platelet factors or extrinsic blood factors are the cause of observed aggregation differences.

Our studies focus on differences between platelets from women and men to aggregate in response to a stimulus of arachidonic acid in the proliferative and secretory phases of the menstrual cycle and before and after exposure to decompression stress. Additionally, differences in platelet thromboxane B₂ production between platelets from women and men were assessed. Washed platelets were used to eliminate sex variations in plasma components.

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Material and methods

Study 1: Platelet aggregation and platelet thromboxane production in proliferative versus secretory phase of menstrual cycle. Twenty-four women were studied, 12 in the proliferative phase and 12 in the secretory phase of the menstrual cycle. The volunteers were free of any acute or chronic disease process. All volunteers denied the use of any medications, specifically, steroids and prostaglandin synthetase inhibitors in the 2-month period before sampling. All volunteers reported regular menstrual cycles of approximately 28day intervals with normal flow. Those volunteers whose cycle were in the proliferative phase had blood taken during cycle days 6 through 12, and those in the secretory phase had blood taken from cycle days 18 through 24 on the basis of the beginning of the last menstrual period. All volunteers were between the ages of 20 and 43 years with a mean age of 29 years. The mean age of volunteers in the proliferative phase was 31 years and in the secretory phase was 27 years. All samples were obtained from a vein in the antecubital area of the arm between 8 and 8:30 AM in the nonfasting state. This study was approved by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions, Baltimore.

Study 2: Effect of decompression stress on platelet aggregation and platelet thromboxane production in women and men. Six women and six men volunteers were recruited from flight crews on active duty in the United States Air Force at Andrews Air Force Base, Maryland. Ages of volunteers ranged from 28 to 41 years with a mean age for women of 36 years and a mean age for men of 31 years. All subjects were medically qualified for flight operations. No differentiation as to proliferative or secretory phase of the menstrual cycle was made in the women of the volunteer group. All blood samples were taken from a vein in the antecubital area of the arm at 8 AM while the subjects were in a nonfasting state. Immediately after the preflight blood sampling the subjects entered a hypobaric chamber at the Physiological Training Unit at Andrews Air Force Base and a modified type III hypobaric chamber flight was accomplished. The flight lasted for 1 hour, during which time all subjects were breathing 100% oxygen by mask until the final recompression at the end of the flight. The flight consisted of an initial decompression to 5000 feet, before which all subjects completed 30 minutes of oxygen breathing to assure denitrogenation. After this a recompression back to sea level was accomplished to correct any oxygen mask malfunctions or trapped gas problems. The chamber was then decompressed to 35,000 feet to create a decompression stress. After stabilization at 35,000 feet the chamber was recompressed to 25,000 feet, where it was leveled, and each volunteer participated in a hypoxia exercise consisting of removal of the oxygen mask until symptoms of hypoxia were experienced. Oxygen masks were then replaced, and after a short period of stabilization the chamber was further recompressed to 18,000 feet, at which point all volunteers were switched to normal oxygen (20% oxygen) for the remainder of the recompression back to sea level. Immediately after return to sea level each volunteer exited the chamber and a second blood sample was obtained. This study was approved both by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions and by the Air Force Human Use Committee, Office of the Surgeon General, Bolling Air Force Base, Washington, D.C.

Platelet aggregation. From each volunteer in both studies a 20 ml blood sample was drawn into a plastic syringe with an 18-gauge needle. Nine milliliters of the whole blood sample was placed into each of two plastic centrifuge tubes, to which was added 1 ml of buffered sodium citrate at room temperature. An immediate hematocrit was accomplished on the remaining whole blood sample.

The citrated blood was centrifuged at 21° C at 190g for 15 minutes. After centrifugation, the platelet-rich plasma was drawn off, the amount recorded, and the remaining packed cells were discarded.

The platelet-rich plasma was centrifuged at 21° C at 760g for 15 minutes. The resulting platelet pellet was rinsed twice with a small amount of Tris-buffered saline solution (0.15 mol/L, pH 7.4), with 2.0% disodium ethylenediaminetetraacetate (EDTA). An additional amount (1 to 2 ml) of Tris-buffered saline solution with EDTA was added and the platelet pellet was resuspended by vortex blending. The suspension was diluted by additional Tris-buffered saline solution with EDTA to equal the volume of platelet-poor plasma previously removed.

The resuspended platelets were centrifuged at 21° C at 760g for 15 minutes, after which the supernatant was discarded. The platelet pellet was resuspended with 10% Tris in Hanks' balanced salt solution without calcium to the same volume as before centrifugation. A platelet count was accomplished by making a 1:100 dilution of the washed platelet solution with Tris without calcium. Platelets were counted on an improved Neubauer AO hemocytometer (Buffalo, N.Y.).

The washed platelets from each volunteer were diluted to a mean concentration of 170,000 cells per milliliter and evaluated for aggregation in response to arachidonic acid. All evaluations were accomplished within a 3-hour period from sampling (range, 1 to 3 hours). A 400 μ l sample of washed platelets was placed in each of seven aggregometer cuvettes and preheated

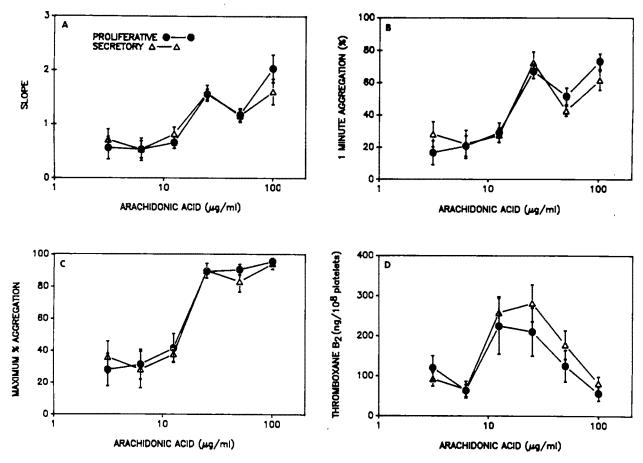


Fig. 1. Aggregation and thromboxane B2 production by isolated platelets from women in proliferative versus secretory stage of cycle.

to 37° C. Then 100 µl of Hanks' balanced salt solution with calcium (1.26 mmol/L) was added to each cuvette and preincubated for 1 minute, after which the arachidonic acid was added in doses ranging from 0 to 100 μg/ml (0, 3.12, 6.25, 12.5, 25, 50, and 100). The aggregation curves produced were then evaluated for slope, aggregation at 1 minute, and maximum aggregation at 5 minutes. After aggregation, all samples were quick frozen at -70° C in a freezer (Revco Scientific, Inc., Asheville, N.C.) and saved for subsequent thromboxane B2 assay.

All frozen samples of washed platelets from study 1 and study 2 were assayed for thromboxane B2 concentration by radioimmunoassay as previously described and validated.19

Data analysis. After completion of the two studies the results were compared between the two groups in study 1 and the two groups in study 2. Data were analyzed by analysis of variance of a nested and repeatedmeasure design.20 For study 1 the repeated measure was dose of arachidonic acid and the nested variable was phase of the menstrual cycle. For study 2 the nested variable was male versus female. In this study there were two repeated-measure variables, namely, arachidonic dose and before versus after decompression stress. The F distribution tests were determined, and data were considered significant at p < 0.05.

Results

Study 1. There was a direct relationship between the concentration of arachidonic acid and all aggregation parameters (Fig. 1, A, B, and C). Thromboxane B₂ production increased with arachidonate doses up to 25 μg/ml. However, at 50 and 100 μg/ml the amount of thromboxane B₂ produced by the platelets was actually less than that produced by those stimulated with 25 μg/ml (Fig. 1, D). In spite of this fact, aggregation occurred at maximal levels at 100 µg/ml. There was no difference in platelet aggregation response to arachidonic acid between females in the proliferative and in the secretory phase of the menstrual cycle (Fig. 1, A, B, and C). Likewise, thromboxane B_2 production by arachidonate-stimulated platelets was not affected by stage of the menstrual cycle (Fig. 1, D).

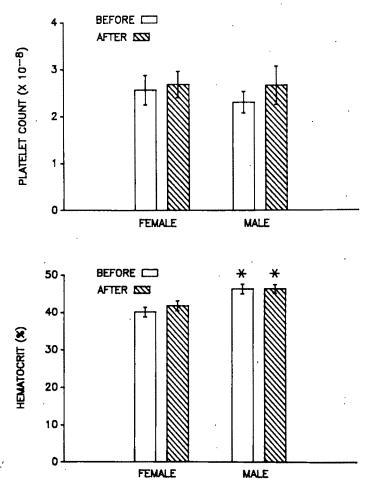


Fig. 2. Platelet count and hematocrit before and after exposure to decompression stress. Males versus females, p < 0.05.

Study 2. In study 2 there was no difference in platelet counts between women and men, but men had a significantly higher hematocrit value (p < 0.05, Fig. 2). Decompression stress did not affect the platelet count or hematocrit.

Platelets from women showed a greater slope in aggregation curve (p < 0.05, Fig. 3) and a greater 1-minute aggregation (p < 0.05, Fig. 4) and maximum aggregation (p < 0.01, Fig. 5) in response to arachidonic acid than did platelets from men. Maximum platelet aggregation was slightly less (6.9%) after decompression stress (p < 0.05, Fig. 5) independent of sex. No other differences in platelet aggregation that were caused by decompression stress were observed (Figs. 3, 4, and 5). Thromboxane B_2 production by isolated platelets was not significantly different between women and men or before or after decompression stress (Fig. 6). However, the same phenomenon of lower thromboxane production in response to the highest concentrations of arachidonic acid was observed.

Comment

The process of platelet aggregation involves a complex cascade of events beginning with platelet membrane receptor exposure and ending with a platelet-platelet bridging by fibrinogen. During this process arachidonic acid from the platelet membrane is converted by the cyclooxygenase pathway to thromboxane A₂. Thromboxane A₂ acts in concert with adenosine diphosphate to recruit circulating platelets and promote aggregation.²¹ The importance of these reactions initially was of interest because of their role in hemostasis. Recently their role in immune and nonimmune defense mechanisms, in embryonic development, and in altered reaction states as a result of sex steroids and external stress has been of increasing concern.

The possibility that platelets might express a sex difference in response to aggregating agents, including sex steroids, and to some forms of stress, such as decompression, has prompted considerable interest to both the basic and clinical researcher, to the aerospace

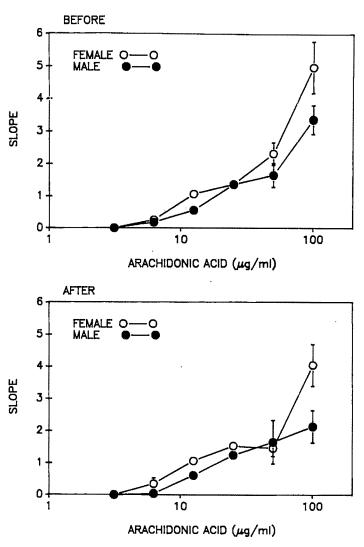


Fig. 3. Slope of platelet aggregation as function of arachidonic acid dose. Males versus females, p < 0.05. Before versus after decompression stress, not significant.

medicine clinician, and to the obstetrician-gynecologist. In the 1960s several articles appeared in support of the idea that platelets from women given estrogen underwent significant behavioral changes, such as increased adhesiveness to a glass surface and increased sensitivity to adenosine diphosphate.22-24 In the 1970s and 1980s, other articles supported the idea that platelets from women showed a greater sensitivity to aggregating stimuli than did platelets from men.13-18 With adenosine diphosphate as an aggregating agent, Johnson et al.13 found that primary aggregation of platelets in young healthy women was significantly increased over values obtained in men, and when epinephrine was used as a stimulant, primary and secondary aggregation also was significantly greater in women than in men. In a later article these investigations17 noted that the sex differences in platelet aggregation in adults most likely were due to an artificially induced difference in plasma calcium levels caused by sex differences in packed cell volume. Roper et al.16 similarly found a difference between aggregation response elicited by adenosine diphosphate for samples from women and men donors and suggested this response was affected to some degree by (1) platelet concentration of the test sample and (2) the time lag from venipuncture to testing. Reading and Rosie¹⁸ have suggested that, though platelets from women have a higher sensitivity than those from men to aggregation by adenosine diphosphate, responsiveness in men increased significantly with age and at a rate greater than that for women, a finding also supported by Johnson et al.13

These data therefore would support an increased sensitivity to aggregation of platelets from women over that in the men; however, the explanation for this dif-

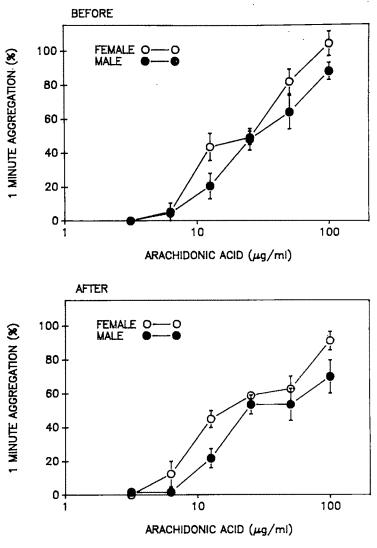
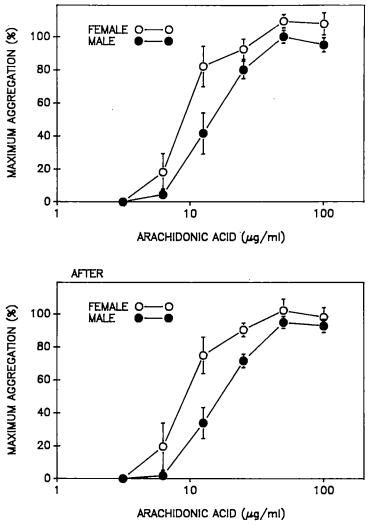


Fig. 4. One-minute aggregation as function of arachidonic acid dose. Males versus females, p < 0.05. Before versus after decompression stress, not significant.

ference is not clear. Only two possibilities for such differences would seem likely: (1) factors intrinsic to the platelet itself (genetic, biochemical) or (2) factors relating to the environment in which the platelets are immersed (plasma factors and/or other blood cells). Most of the reported platelet aggregation studies have been accomplished on whole blood or on platelet-rich plasma; therefore it has been difficult to assess factors of aggregation because of the compounding effect of variations of packed cell volumes, plasma calcium, and other plasma factors on the aggregation process.

In study 1 we have attempted to eliminate some of these variables by using washed platelets to remove plasma and other extrinsic cell factors, focusing on the isolated platelet reaction to a known aggregating agent, arachidonic acid. With this approach our results indicate a direct relationship between concentration of arachidonic acid and all assessed platelet aggregation parameters (slope, 1-minute aggregation, and maximum aggregation), a finding in agreement with the data of others.²⁵ Furthermore, the response was similar in platelets sampled from women during the proliferative and secretory phases of the menstrual cycle. These findings support the idea that sex steroids, or cycle changes in sex steroids, do not directly or acutely alter the ability of isolated platelets to respond to an aggregating stimulus or to produce thromboxane.

Decompression sickness has been associated with increased clotting activity, and there is evidence that increased platelet aggregation leading to intravascular clotting and thrombosis formation may cause some of the symptoms observed in decompression exposure. This idea is supported by the observation that a reduction in platelet count has been noted after decompression in human subjects. Experiments by Jacobs and Stewart, involving severing the tips of tails



BEFORE

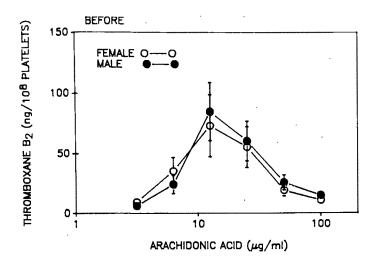
Fig. 5. Maximum aggregation as function of arachidonic acid dose. Males versus females, p < 0.01. Before versus after decompression stress, p < 0.05.

of decompressed rats, revealed a bloody froth issuing from the vessels in the proximal stump of the tail, and on microscopic examination small bubbles surrounded by platelet aggregates were seen. Additionally, experimental decompression in rats has been followed by a loss of circulating platelets and an increase in thrombosis, the extent of which correlated with the severity of decompression symptoms.28

Recently several studies have suggested that decompression stress and decompression sickness occurs more frequently in women than in men.29-31 Bassett29 found that women had a tenfold greater incidence of altitude decompression sickness when compared with that among men. Weien and Baumgartner³⁰ have reviewed 528 cases of decompression sickness resulting from an altitude exposure in either an aircraft or a hypobaric chamber. Their data suggested that the relative risk of decompression sickness necessitating hy-

perbaric therapy was 4.3 times greater for women than for men. An article by Rudge³¹ focused on the relationship of chamber decompression sickness in women related to the menstrual cycle and found a greater incidence at or around the time of menses. These data, as well as a study suggesting an increased potential for decompression stress and decompression sickness in astronauts,32 prompted our study on the effects of decompression stress, produced by a hypobaric chamber flight, on platelet aggregation of washed platelets from women and men.

Our results in study 2, assessing washed platelets from before and after exposure to a decompression stress, showed that platelets from women had a greater slope in aggregation curve, as well as a greater 1-minute and maximum aggregation both before and after the stress exposure. This would suggest a sex difference unrelated to the decompression exposure. Maximum



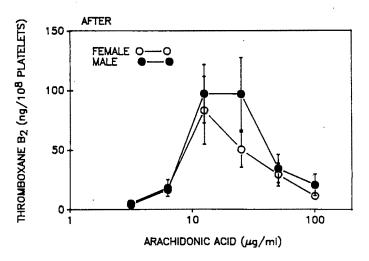


Fig. 6. Thromboxane B2 production by isolated platelets as function of arachidonic acid dose. Males versus females, not significant.

platelet aggregation in women was found to be slightly less after decompression stress (6.9%, p < 0.05). In this study group there was no difference in platelet count or platelet thromboxane production between sexes. Earlier studies²⁵ demonstrated an inhibition in aggregation with arachidonic acid doses >100 µg/ml, and for this reason we did not use larger doses. Surprisingly, we found a smaller amount of thromboxane release from platelets with the highest doses of arachidonic acid used (50 and 100 µg/ml). In spite of the lower amount of thromboxane produced, maximum aggregations still occurred. Thus, at these doses, factors other than thromboxane are responsible for the aggregation. These factors might be other arachidonate products or direct effects of arachidonic acid. Direct effects of nonmetabolized arachidonate have been demonstrated on calcium release by macrophages.33

· Our data with isolated washed platelets indicate that

there is an increased sensitivity of platelets from women compared with men to aggregation as exhibited in each of the aggregation parameters. This difference was observed both before and after an exposure to decompression stress; therefore it is unlikely that decompression itself initiates a gender difference. It is also unlikely that thromboxane production by the platelet plays a role in the gender difference since the production rates between platelets from women and men were statistically similar.

In conclusion, platelet aggregation and thromboxane production appear to have physiologic relationships to many aspects of reproductive medicine. The possibility that platelets aggregate differently during the menstrual cycle or that the thromboxane production is altered in relationship to changes in sex steroids is not supported by our studies. Additionally, our studies support a sex difference in aggregation that is not altered by external factors such as decompression sickness. This is of importance to the obstetrician-gyne-cologist who is assigned the medical care of the increasing numbers of women competing in the aerospace industry.

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Tryptophan and neutral amino acids in premenstrual syndrome

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An abnormality of the serotonin system may play a role in the genesis of the symptoms of premenstrual syndrome, especially those related to mood and appetite. Whole blood and platelet uptake of serotonin are decreased during the luteal phase in women with premenstrual syndrome. The ratio of L-tryptophan (the amino acid precursor of serotonin) to the sum of the other neutral amino acids that compete for the same protein carrier cerebral uptake mechanism has been suggested to reflect central L-tryptophan levels and resultant serotonin levels in the brain. We evaluated the ratio of plasma L-tryptophan to the sum of five competing neutral amino acids in women with premenstrual syndrome and in controls. There were no significant differences between groups or across time, suggesting that it is unlikely that the aberrations of the serotonergic system in women with premenstrual syndrome are due to saturation of the tryptophan-carrying protein. (AM J OBSTET GYNECOL 1991;165:1830-3.)

Key words: Neutral amino acids, premenstrual syndrome

Premenstrual syndrome (PMS) is a psychoneuroendocrine disorder of uncertain cause. Prominent symptoms include the cyclic appearance of marked depression, mood swings, irritability, anger, decreased energy, fatigue, and hyperphagia, specifically cravings for carbohydrate-rich foods; these symptoms may be of sufficient severity to resemble those of affective disorder. However, the presence of a postmenstrual symptomfree week and failure to document biologic markers for depression in well-screened females with PMS suggest that PMS and major affective disorder are discrete entities.¹⁻⁵

There are numerous studies suggesting a disorder of serotonin (5-hydroxytryptamine) metabolism in depression.4.5 Similarly, in subjects with PMS the concentration of 5-hydroxytryptamine in whole blood and the platelet uptake of 5-hydroxytryptamine are diminished during the luteal phase.⁶⁻⁸ The exact mechanism by which a deficiency of the serotonin system may play a role in the genesis of the symptoms of PMS is not yet known. L-Tryptophan, the amino acid precursor of 5hydroxytryptamine, and other large neutral amino acids (e.g., tyrosine, valine, leucine, isoleucine, and phenylalanine) compete for the same saturable carrier protein for transport across the blood-brain barrier. 9 It has been proposed that the ratio of plasma L-tryptophan to the sum of those competing amino acids reflects central L-tryptophan levels and resultant 5-hydroxytryp-

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tamine levels in the brain. 9-11 We therefore elected to evaluate the ratio of plasma L-tryptophan to the sum of the five competing amino acids during the follicular and luteal phase in well-screened subjects with PMS and in controls.

Material and methods

Selection of subjects. Subjects with PMS and controls were recruited through newspaper advertisements. Eligibility criteria for subjects with PMS have been previously described in detail¹²; they were required to fulfill the *Diagnostic and Statistical Manual*, third edition, revised,¹³ criteria for late luteal phase dysphoric disorder. Prospective subjects completed a baseline daily symptom diary for 2 months before and during the study. The PMS diary consisted of 11 symptoms, outlined in Table I. All subjects had regular menses with a biphasic basal body temperature curve.

After noneligible subjects were excluded, 18 PMS subjects and 16 control subjects remained for evaluation. Subjects were instructed to abstain from nutritional or hormonal supplements, vigorous exercise, or unusual diets for at least 1 month before phlebotomy. The mean age was 32.4 years and 27.2 years for PMS and control subjects, respectively. Informed consent was obtained in all cases, and the study was approved by the Human Subjects Protection Committee at the University of California at Los Angeles.

Assays. During the third month of the study subjects had venipuncture at 8 AM, after a 12-hour fast. Phlebotomy was performed once on days 8 to 10 of the cycle (day 1 was considered the first day of bleeding) and once on days 24 to 26 of the cycle (10 to 12 days after the midcycle elevation of the basal body temperature curve). Blood was obtained for determination of

Table I. Scores (mean \pm SE) on daily diary for PMS and control subjects during follicular and luteal phase sampling intervals

	PMS subjec	ts (N = 18)	Control subjects $(N = 16)$		
Diary variable	Follicular (mean ± SE)	Luteal (mean ± SE)	Follicular (mean ± SE)	Luteal (mean ± SE)	
Avoid social activity	1.29 ± 0.12	3.51 ± 0.27*	1.07 ± 0.04	1.20 ± 0.08	
Decreased work	1.51 ± 0.21	$3.39 \pm 0.30*$	1.29 ± 0.10	1.30 ± 0.10	
Edema	1.44 ± 0.07	$4.04 \pm 0.24*$	1.05 ± 0.05	1.27 ± 0.10	
Depression	1.51 ± 0.16	$3.40 \pm 0.31*$	1.24 ± 0.13	1.17 ± 0.06	
Anxiety	1.49 ± 0.14	$3.59 \pm 0.27*$	1.17 ± 0.09	1.21 ± 0.10	
Mood swings	1.34 ± 0.12	$3.71 \pm 0.28*$	1.17 ± 0.10	1.25 ± 0.12	
Anger	1.36 ± 0.12	$3.71 \pm 0.29*$	1.25 ± 0.14	1.31 ± 0.13	
Food cravings	1.51 ± 0.17	$3.64 \pm 0.30*$	1.28 ± 0.14	1.41 ± 0.06	
Decreased energy	$1.68 \pm 0.21 \dagger$	$3.69 \pm 0.22*$	1.23 ± 0.09	1.31 ± 0.10	
Headache	$1.69 \pm 0.20 \dagger$	$2.26 \pm 0.23*$	1.13 ± 0.05	1.08 ± 0.04	
Breast tenderness	1.06 ± 0.06	$2.86 \pm 0.33*$	1.01 ± 0.01	1.37 ± 0.13	

^{*}Denotes significant differences between PMS and control subjects during luteal phase assessment (p < 0.001).

plasma tryptophan, tyrosine, valine, isoleucine, leucine, and phenylalanine. The assays were performed by Aatron Medical Services (Santa Monica, Calif.).

The deproteinization procedure involved the addition of 400 µg 0.5% sodium dodecyl sulfate to 400 mm³ plasma. The mixture was incubated at room temperature (25° C) for 15 minutes, and the tube was mixed every 3 to 5 minutes. Aliquots of 500 µl of 10% sulfosalicylic acid was then added and gently mixed by hand until homogeneous. Then 700 µl of 0.5 mol/L lithium hydroxide was added, and the mixture was centrifuged for 20 minutes at 2500 rpm at 5° to 10° C. A 400 µl aliquot of supernatant was transferred into a plastic tube to which 400 µl of 2.5 mmol/L s-2-aminomethyl-L-cystine was added. The mixture was then centrifuged for 15 minutes and loaded into a sample loop for injection into the ion-exchange highperformance liquid chromatography amino acid analyzer (model 6300, Beckman Instruments, Inc., Fullerton, Calif.). Ion-exchange high-performance liquid chromatography was used for separation and ninhydrin reaction allowed for visible colorimetry for detection of the above-noted amino acids. The intraassay and interassay variations for amino acid concentrations were <2%.

Statistical analysis. The sum of the five competing amino acids (tyrosine, valine, isoleucine, leucine, and phenylalanine) was determined, and a ratio was computed between L-tryptophan and the five competing amino acids. The ratio was determined for the follicular and luteal phase sampling intervals in the PMS and control subjects. Plasma L-tryptophan to competing amino acid ratios were computed for each menstrual cycle phase as noted above, and comparisons were made between groups and across time on these variables with analysis of variance.

Daily diary scores were calculated by obtaining the

Table II. Tryptophan-to-competing amino acid ratios (mean ± SE) during follicular and luteal phases for PMS and control subjects

	Follicular phase ratio	Luteal phase ratio
PMS $(N = 18)$	0.070 ± 0.013	0.074 ± 0.008
Control $(N = 16)$	0.108 ± 0.023	0.071 ± 0.014

mean and standard deviations for each diary symptom for the day before, the day of, and the day after each venipuncture. The mean scores for each of the symptoms during the follicular and luteal assessment phases of the cycle were then calculated for the PMS and control groups, respectively. Within-group, across-group, and between-group comparisons in the symptom scores were performed with the Student t test and analysis of variance, where appropriate, to test the differences between the means and for a group-by-phase interaction.

Results

The mean scores on the daily diary are shown in Table I. Subjects with PMS and controls have similar scores during the follicular phase with only the PMS subjects showing significant premenstrual exacerbation of symptoms. There were no significant differences in height or weight between PMS and control subjects.

The mean L-tryptophan—to—competing amino acid ratios during the follicular and luteal phase for PMS and control subjects were outlined in Table II. There were no significant differences between groups or across time in the L-tryptophan—to—competing amino acid ratios.

Comment

Decreased serotonergic activity and decreased platelet uptake of 5-hydroxytryptamine have been reported

[†]Denotes significant differences between PMS and control subjects during follicular phase assessment (p < 0.05).

in patients with endogenous depression.^{4, 5, 14} Similar findings have been noted in the luteal phase in women with PMS.⁶⁻⁸ The importance of the serotonergic system in regulating mood and behavior is further supported by studies in which nonhuman primates that were administered pharmacologic agents that depleted 5-hydroxytryptamine and human subjects who were restricted to tryptophan-deficient diets manifested anger, aggression, social withdrawal, and increased food intake, which resemble the affective and appetitive derangements of PMS.¹⁵⁻¹⁷ Furthermore, treatment with the serotonergic agonist fenfluramine has been reporteed to alleviate PMS symptoms.¹⁸

The principal objective of the present study was to test the hypothesis that diminution of central nervous system L-tryptophan uptake in women with PMS could account for the symptoms seen in the luteal phase in these patients. We tested the hypothesis by estimating, in well-selected subjects with PMS and in control subjects, the ratio of plasma L-tryptophan to the sum of the other large neutral amino acids that compete for the same carrier protein for transport into the central nervous system.

The serotonin levels in the central nervous system are dependent on local conversion of L-tryptophan to 5-hydroxytryptamine, as the latter does not cross the blood-brain barrier. Carbohydrate-rich diets have been shown to increase, and protein-rich diets to decrease, brain L-tryptophan levels in the rat. L-Tryptophan loading has been shown to increase brain tryptophan in dogs and to augment the amino acid metabolite of serotonin, 5-hydroxyindole acetic acid, in the cerebrospinal fluid of both dogs and human subjects. An interesting feature of PMS is the craving for high-carbohydrate diets at the time of maximal symptoms in the luteal phase.

The limiting factor controlling central nervous system uptake of L-tryptophan is the transport protein that carries L-tryptophan and other large neutral amino acids across the blood-brain barrier. Binding to this carrier is competitive and saturable, and therefore central nervous system uptake of L-tryptophan depends not only on peripheral L-tryptophan concentration but also on the levels of the other competing amino acids. Various studies have suggested that the ratio of plasma L-tryptophan to the sum of these competing amino acids predicts central L-tryptophan and resultant serotonin levels in the brain. This ratio also has been found to be decreased in patients with major depressive disorders and depression associated with alcoholism. 23-25

In this investigation we have found that well-selected patients with PMS showed no difference in the luteal phase L-tryptophan-to-competing amino acid ratios when compared with follicular phase ratios or ratios measured in control subjects during the luteal phase. These findings suggest that there is adequate availability of L-tryptophan to the central nervous system in patients with PMS and that if a deficiency in the serotonergic system exists in the luteal phase of patients with PMS it is unlikely to be related to inadequate substrate. It is possible that excess consumption of simple carbohydrates by subjects with PMS in the late luteal phase could have elevated the tryptophan-to-competing amino acid ratios obtained in PMS subjects and thus compensated for an alteration in the ratio that otherwise may have been present. The use of the 12hour fasting sample, however, should have minimized this potential problem.9

This is the first investigation of the L-tryptophan—to—competing amino acid ratio in subjects with PMS. Unlike the results obtained in PMS patients, this ratio has been noted to be decreased in individuals with affective disorders. ^{25–25} This difference between these two clinical conditions again highlights earlier reports that PMS is not a subset of cycling affective disorder. Further studies are needed to identify the differences, if any, in the conversion of L-tryptophan to 5-hydroxy-tryptamine in the central nervous system of these patients, because substrate availability does not seem to be an important factor.

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The role of prostaglandins in detrusor instability

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To study the role of prostaglandin production by detrusor muscle in women with idiopathic primary detrusor instability, biopsies of detrusor muscle were obtained from 20 women. Nine women had detrusor instability, and 11 women had stable bladders. Prostaglandin $F_{2\alpha}$ and the stable metabolites of prostacyclin and thromboxane A_2 , namely, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 , respectively, were measured after in vitro incubations of detrusor muscle for 3 hours. A significant reduction in the production of 6-keto-prostaglandin_{1\alpha} in women with detrusor instability was noted. There were no differences in the production of prostaglandin $F_{2\alpha}$ and thromboxane B_2 , between women with stable and unstable bladders. These results suggest for the first time that women with idiopathic primary detrusor instability may have a deficient production of prostacyclin. (AM J OBSTET GYNECOL 1991;165:1833-6.)

Key words: Prostaglandins, detrusor instability, bladder prostacyclin

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Primary instability of the detrusor muscle is a condition of unknown cause. In some cases the condition is secondary to central nervous system pathology and is called detrusor dyssenergia.^{1, 2} In most cases it is an idiopathic condition for which treatment has not been satisfactory.^{3, 4}

Prostaglandins are known to play a major role in

Table I. Prostaglandin production by detrusor muscle biopsy specimens from patients with stable and unstable bladders

Incubation – time		6-Keto-PGF _{1a} (ng/mg wet weight of tissue)				
	Stable		Unstable		Stable	
	· Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
1 hr 2 hr 3 hr	0.258 ± 0.121 0.483 ± 0.212 0.914 ± 0.397	0.386-0.096 0.699-0.253 1.343-0.419	0.197 ± 0.098* 0.306 ± 0.165* 0.496 ± 0.238*	0.303-0.094 0.491-0.197 0.773-0.221	3.07 ± 1.14 2.93 ± 0.96 2.47 ± 0.60	4.217-2.766 4.804-2.075 3.113-2.006

^{*}Not significantly different when compared with stable detrusor group.

smooth muscle contraction and to exert an effect on the detrusor muscle as well. In vitro studies have shown that both prostaglandin F2a (PGF2a) and prostaglandin E2 (PGE2) significantly enhance detrusor contractions.5 In vivo studies have shown that prostaglandins, administered either directly to the bladder or systemically, cause effective detrusor contractions and improve bladder function after gynecologic surgery.6,7 Antiprostaglandins have been used in women with uncontrolled bladder contractions and detrusor instability.8 The use of indomethacin for treatment of idiopathic detrusor instability has not gained popularity because of suboptimal efficacy and the significant side effects encountered.8

Therefore our current work was undertaken to assess prostaglandin production by detrusor muscle in women with idiopathic primary detrusor instability. More specifically, we wished to determine the production of prostacyclin, a relaxing, vasodilatory prostaglandin.

Material and methods

Twenty women scheduled to undergo abdominal hysterectomy because of uterine leiomyomas, with or without bladder neck suspension, agreed to participate in this study. The mean age of these women was 51 (range 31 to 76) and mean parity was 3 (range 1 to 9). Eleven of these women were premenopausal and nine were postmenopausal. All postmenopausal women who were not on a regimen of estrogen replacement therapy received such therapy in the form of conjugated estrogens (Premarin, Ayerst Laboratories, New York), 0.625 mg/day for 2 weeks before evaluation and continued this treatment until operation. All women underwent surgery within 4 weeks of evaluation. At surgery, no woman had inflammatory conditions such as salpingitis, pelvic abscess, or endometriosis. All premenopausal women were scheduled to undergo operation at midcycle, although the exact day could not always be determined.

All women underwent preoperative urethrocystometry with a Disa 2100 multichannel electrophysiologic recorder (Dantek Lab, Hoboken, N.J.) and microtip pressure transducer (Millar 60 K 22 microtip catheter, Millar Instrument Co., Houston) for concomitant detrusor and urethral pressure recordings). All tests were carried out with the patients in the standing position with rapid water filling (at a rate of 60 ml/min). Nine of the women in this series had detrusor instability, and 11 women had stable bladders. All with detrusor instability had motor detrusor instability as defined by the International Continence Society.9 The nine with detrusor instability had repeated uninhibited bladder contractions, of >15 cm of water above baseline, starting at bladder volumes of 120 to 275 ml. The 11 women with a stable bladder (International Continence Society definition9) in this series had no detectable bladder contractions on rapid bladder filling in the standing position and had a baseline bladder pressure increase from the beginning of urodynamic testing to maximal cystometric capacity of <15 cm of water. The maximal cystometric capacity of the 11 women with stable detrusor ranged from 320 to 585 ml. Both groups (stable and unstable) were comparable with respect to mean age, parity, and menopausal status. There was no correlation between age and findings of detrusor instability. Mean age of the nine women with detrusor instability was 52 years and that of the 11 women with stable bladder was 50.5 years.

At the time of operation, two punch biopsy specimens were taken from the dome of the bladder. These samples $(1 \times 1 \text{ cm})$ were obtained deep in the detrusor muscle but excluded the bladder mucosa. The biopsy site was then closed with assured 2-0 Vicryl (Ethicon, Summerville, N.J.) sutures, and the integrity of the bladder was assumed by instillation of methylene blue. All specimens were examined histologically for the presence of detrusor muscle fibers.

Bladder incubation procedure. After surgical removal, each specimen of bladder tissue was immediately placed in cold Dulbecco's minimal essential medium (Gibco Laboratories, Santa Clara, Calif.) and incubated within 20 minutes after its removal. The

 $[\]dagger p < 0.01$ compared with the stable detrusor group.

 $[\]pm p < 0.05$ compared with the stable detrusor group.

	6-Keto-PGF _{1a} (ng/mg wet weight of tissue) Unstable		TxB2 (ng/mg wet weight of tissue)				
Unsi			Stable		ble		
Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range		
0.62 ± 0.12† 0.59 ± 0.17‡ 0.42 ± 0.91‡	0.767-0.506 0.769-0.303 0.536-0.284	$\begin{array}{c} 0.027 \pm 0.019 \\ 0.039 \pm 0.020 \\ 0.031 \pm 0.017 \end{array}$	0.049-0.011 0.067-0.101 0.053-0.019	0.048 ± 0.056* 0.51 ± 0.029* 0.049 ± 0.051*	0.104-0.072 0.844-0.301 0.103-0.040		

muscle tissue was first dissected free of serosa and minced finely with a scalpel, weighed rapidly, and placed in a vial containing 2 ml Dulbecco's minimal essential medium. This mixture was then placed in a Dubnoff incubator in an atmosphere of 95% oxygen and 5% carbon dioxide at 37° C for 3 hours. A 0.5 ml aliquot of the medium was taken after 0.5, 1, 2, and 3 hours of incubation to measure prostaglandin levels. Each time an aliquot of the incubated medium was removed, it was replaced with 0.5 ml of fresh Dulbecco's minimal essential medium, to maintain a total incubation volume of 2 ml. The aliquots were stored at -20° C until processed within 1 month following the incubation.

Measurement of prostaglandin levels. Levels of $PGF_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF_{1\alpha}), and thromboxane B2 (TxB2) were measured by specific radioimmunoassays (RIA). PGF_{2α} was measured with a [3H] PGF2a RIA kit (Advanced Magnetics, Inc., Cambridge, Mass.) with a modified procedure. The modifications included (1) the use of a 0.05 ml aliquot of sample for assay, (2) addition of approximately 2000 dpm to each sample as internal standard to follow procedural losses; and (3) reconstitution of the extract in 1 ml of the assay buffer and use of 0.05 ml of the solution in duplicate for RIA and 0.5 ml to determine the procedural losses.

6-Keto-PGF_{1α} and TxB₂ were quantitated by RIA, after their extraction from separate 0.1 ml aliquots of samples. Before extraction, each sample was diluted with 0.4 ml of assay buffer (phosphate-gelatin buffer consisting of 0.1 mol/L sodium phosphate, pH 7.0, and 0.15 mol/L sodium chloride and containing 0.1% (wt/vol) of Knox unflavored gelatin and sodium azide). To this mixture were added approximately 2000 dpm of the appropriate tritiated prostaglandin (either ³H-6-keto-PGF_{1α} [Amersham Corporation, Arlington Heights, Ill.; specific activity 157 Ci/mmol] or 3H-TxB2 [DuPont NEN Research Products, Boston; specific activity 180 Ci/mmol]) in 0.1 ml of assay buffer. After the mixture was blended in a vortex, its pH was adjusted to 3 to 3.5 with formic acid. The prostaglandins were then extracted with pretreated Bond Elut C18 columns (3 ml; Analytichem International, Harbor City,

Calif.) by sequential elution with 2 ml water, 2 ml benzene, and 3 ml ethyl acetate. The latter solvent, which contained the prostaglandins, was evaporated to dryness under nitrogen at 37° C, and the residue was redissolved in 1 ml of methanol/assay buffer [1:20 vol/vol]. From this solution a 0.5 ml aliquot was taken to determine procedural losses and aliquots of 0.02, 0.05, and 0.1 ml were used for RIA. The 6keto-PGF_{1a} RIA uses ³H-6-keto-PGF_{1a}, in conjunction with anti-6-keto-PGF₁₀, serum (a gift from Dr. Carlo Patrono, Universita Cattolica del Sacro Cuore, Rome), whereas the TxB2 RIA uses 3H-TxB2 in conjunction with anti-TxB₂ serum (The Upjohn Co., Kalamazoo, Mich.). After a 16- to 18-hour incubation at 4° C the free and antibody-bound prostaglandins were separated by adding 0.6 ml of a dextran-coated charcoal (Norit A, Sigma Chemical Co., St. Louis) suspension (0.5% wt/vol charcoal and 0.05% wt/vol dextran).

Data analysis. Data are reported as mean ± SD and are expressed in nanograms per milligram of wet weight of tissue. The two-tailed t test was used for statistical analysis of the data.

Results

Nine of the women in this series had primary idiopathic detrusor instability and 11 women had stable bladders, as assessed by preoperative urodynamics. This information was not made available to the laboratory at the time of incubation or prostaglandin analysis.

There was a progressive increase in the production of $PGF_{2\alpha}$ by both stable and unstable groups during the 3 hours of incubation (Table I). At the end of 3 hours, the $PGF_{2\alpha}$ production was 3.5- and 2.5-fold greater compared with the first hour in the stable and unstable groups, respectively. However, there was no significant difference between the two groups in the production of PGF_{2α} at either 1, 2, or 3 hours after incubation. Essentially no change in TxB2 production by either the stable or unstable detrusor group was observed between the first and third hours of incubation. At the end of the first hour of incubation, the TxB2 production was almost twofold higher in the unstable detrusor group compared with the stable one. However, after the second and third hours of incubation, no significant differences were observed between the two groups.

A slight decrease was observed in 6-keto-PGF_{1α} production by both the unstable and stable groups during the 3-hour incubation period. The production of 6-keto-PGF_{1α}, however, was fivefold to sixfold greater in the incubates of patients in the stable detrusor group as compared with those from patients with detrusor instability. These differences were significant (p < 0.05) (Table I).

No correlations were evident between any of the prostaglandin measurements and either age or menopausal status.

Comment

The results of this study indicate that women with detrusor instability had a significant reduction in the production of 6-keto-PGF $_{1\alpha}$ and an insignificant change in the production of PGF $_{2\alpha}$ and TxB $_2$ by detrusor muscle.

Detrusor instability may at times be secondary to urethral abnormality, which leads to increased sensory input from the urethra, resulting in bladder contractions.10 In women with primary detrusor instability in which the bladder contraction is not preceded by any urethral pressure changes, the best strategy for treatment is to stabilize the bladder by preventing excessive contractions.10, 11 Various medical treatment methods, such as anticholinergics with or without calcium channel blockers, have been advocated,12 in spite of the fact that these treatments do not achieve a high cure rate. In the past other investigators have suggested the use of antiprostaglandins such as indomethacin on the premise that prostaglandins such as PGF_{2α} stimulate smooth muscle contractions.8 This treatment proved to be somewhat beneficial for a minority of patients, but in these women it was often discontinued because of side effects and less than optimal efficacy.8

These and other treatments have not been successful in part because of the empiric nature of the therapy and because the primary cause of detrusor instability is unknown. Prostaglandins are known to stimulate smooth muscle contraction at multiple sites in the body, including the detrusor muscle. Because only the stimulatory effects of PGF_{2a} and TxA₂ on the detrusor have been considered as causes of detrusor instability, we hypothesized that a dysregulation in the production of prostacyclin also could be responsible. In this setting the relaxing effect of prostacyclin on smooth muscle fibers may be aberrant.

Women with detrusor instability in this study had significantly decreased production of 6-keto-PGF_{1 α}, whereas both PGF_{2 α} and TxB₂ levels were normal. Therefore suppressing all prostaglandins in women with detrusor instability would be expected to inhibit

both the relaxing and contractile prostaglandins and the relaxing effects of prostaglandins to a minimum level. These preliminary data suggest that in women with idiopathic primary detrusor instability decreased prostacyclin production results in an abnormality in the balance between contractile and relaxing influences. This in turn may be a primary defect or a secondary effect of an intrinsic abnormality of the bladder. The findings of a decreased prostacyclin/TxA2 ratio in women with detrusor instability may further suggest an abnormality in bladder vascular tone. This decreased detrusor blood flow or perfusion also may be implicated in this pathophysiologic condition. Therefore it may be reasonable to consider treating patients by enhancing the production of prostaglandin rather than by using nonspecific antiprostaglandin agents. Whether this strategy will result in successful treatment of women with primary detrusor instability remains to be established.

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Is 11β-hydroxyandrostenedione a better marker of adrenal androgen excess than dehydroepiandrosterone sulfate?

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To determine whether the adrenal androgen 11β-hydroxyandrostenedione is a more sensitive and specific marker than dehydroepiandrosterone sulfate, we compared these serum androgens in 81 women with anovulatory hyperandrogenism before treatment, after corticotropin and corticotropin-releasing-factor stimulation, and after short- and long-term dexamethasone suppression. Of all subjects, 65% and 57% had elevated levels of 11β-hydroxyandrostenedione (>2.0 ng/ml) and dehydroepiandrosterone suifate (>2.8 μg/ml), respectively. However, 11β-hydroxyandrostenedione and dehydroepiandrosterone sulfate levels did not correlate in either the women with hyperandrogenism (r = 0.12) or the 26 normal women (r = 0.29). After 0.25 mg corticotropin was administered intravenously (n = 16), 11β-hydroxyandrostenedione increased by 157% ± 53% (mean ± SEM), whereas dehydroepiandrosterone sulfate, androstenedione, dehydroepiandrosterone, and cortisol increased by $6\% \pm 2\%$, $46\% \pm 10\%$, $416\% \pm 80\%$, and $2326\% \pm 371\%$, respectively. After intravenous administration of 100 µg corticotropin-releasing factor to eight patients, the percent change from baseline level to peak was 148% \pm 26%, 24% \pm 5%, 61% \pm 15%, 117% \pm 15%, and 116% \pm 18% for 11β-hydroxyandrostenedione, dehydroepiandrosterone sulfate, androstenedione, dehydroepiandrosterone, and cortisol, respectively. After 2 mg dexamethasone for 3 days (n = 10), 11β -hydroxyandrostenedione, dehydroepiandrosterone sulfate, androstenedione, and testosterone were suppressed by 95% ± 2%, $74\% \pm 3\%$, $51\% \pm 9\%$, and $32\% \pm 9\%$, respectively. Suppression with 0.5 mg dexamethasone for 3 months lowered 11β-hydroxyandrostenedione and dehydroepiandrosterone sulfate levels equally by 50% ± 14% and 62% ± 12%, respectively. 11β-Hydroxyandrostenedione is a useful marker of adrenal androgen secretion with a calculated sensitivity and specificity greater than that of dehydroepiandrosterone sulfate. The greater sensitivity of 11β-hydroxyandrostenedione over dehydroepiandrosterone sulfate to adrenal stimulation and suppression suggests its unique diagnostic use. (AM J OBSTET GYNECOL 1991;165:1837-42.)

Key words: 11β-Hydroxyandrostenedione, dehydroepiandrosterone sulfate, adrenal androgens, hyperandrogenism

It is often unclear whether the source of increased androgen production in women with hyperandrogenism is the ovary, the adrenal gland, or increased peripheral conversion of androgen precursors. In particular the role of the adrenal glands in the development of hyperandrogenism remains to be established.

Although dehydroepiandrosterone sulfate (DHEAS) has been used as a marker of adrenal androgen production for some time, the concept that DHEAS exclusively reflects adrenal androgen activity has been chal-

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lenged because a significant source of DHEAS may be derived from the ovary by direct glandular secretion, hepatic sulfurylation of DHEA originating from the ovary, or both. Using selective catheterization techniques, Moltz et al.1 found significant peripheral-ovarian gradients for DHEAS in two of eight healthy women with ovulatory cycles. Vaitukaitis et al.2 showed that peripheral DHEAS levels in normal men and women increased progressively with long-term administration of exogenous corticotropin, but failed to respond to shortterm corticotropin stimulation. In contrast, peripheral DHEA levels exhibited a 20- to 100-fold rise when corticotropin was administered on a short-term basis. The discrepancy in the response of DHEAS to short- and long-term administration of corticotropin could be explained if DHEA were peripherally converted to DHEAS.

There is evidence that 11β-hydroxyandrostenedione (11β-A) may serve as a valuable marker of adrenal androgen production in women with hyperandrogen-

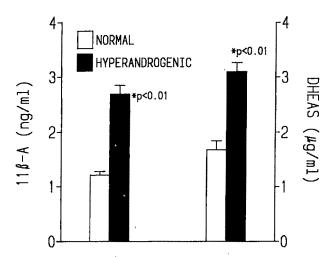


Fig. 1. Serum levels (mean \pm SEM) of 11 β -A and DHEAS in normal women (n=26) and women with hyperandrogenism (n=81).

ism.^{5, 4} 11 β -A is the third most abundant steroid secreted by the adrenal gland and is present in nanogram amounts in blood. However, comparative studies evaluating 11 β -A and DHEAS as biochemical markers of adrenal androgen excess in women with hyperandrogenism have not been reported.

The objectives of this study were (1) to determine whether 11β -A is a more sensitive and specific marker of adrenal androgen excess than DHEAS and (2) to compare the responses of these markers with adrenal stimulation and suppression in women with hyperandrogenism.

Material and methods

Subjects. Study subjects consisted of 26 normal women and 81 women with hyperandrogenism. The women with hyperandrogenism were a mixed group of patients who were initially seen with complaints of androgen excess and who had elevations in serum levels of androstenedione, testosterone, or both. Ages of the normal women ranged from 18 to 30 years; the ages of the women with hyperandrogenism were 18 to 28 years. The body weights of the normal women and the women with hyperandrogenism were $112\% \pm 5\%$ and $117\% \pm 24\%$ (mean \pm SD), respectively, of the ideal body weight.

Treatment and sampling. Sixteen women with hyperandrogenism were treated intravenously with 0.25 mg α^{1-24} -corticotropin (cosyntropin). Blood samples were collected before treatment and 30-minutes after treatment.

Eight women with hyperandrogenism were treated intravenously with 100 μg of ovine corticotropin-releasing factor (Cambridge Research Biomedicals, Ltd., Cambridgeshire, England). Blood sampling was carried out at 0, 30, 60, 90, and 120 minutes after treatment.

Another 24 women with hyperandrogenism were treated with 2 mg of dexamethasone daily for 3 days. Blood was drawn before treatment and after 3 days of dexamethasone.

Finally, seven women with hyperandrogenism were treated with 0.5 mg of dexamethasone daily for 3 months. Blood samples were collected before and after 3 months of dexamethasone.

All blood samples were collected between 8 AM and 10 AM.

Assays. 11\beta-Hydroxyandrostenedione was measured in serum as previously described by Fiet et al.5 In brief, 0.2 ml of serum, to which 1000 to 1200 disintegrations per minute of tritiated 11β-A was added to monitor procedural losses, was extracted with diethyl ether; the extract was subjected to celite column partition chromatography. Elution of 11B-A was carried out with 70% (vol/vol) benzene in isooctane. The 11β-A fraction was redissolved in 0.7 ml of assay buffer; 0.2 ml aliquots were taken in duplicate for quantification by radioimmunoassay (RIA), and a 0.2 ml aliquot was counted to determine procedural losses. The 11β-A RIA uses tritiated 11β-A as radioligand, and an antiserum raised in rabbits against 68,118-dihydroxy-4-androstene-3,17-dione-6β-hemisuccinate-bovine serum albumin. Both the radioligand and antiserum were prepared as described previously by Putz et al.6 The linear range of the standard curve was 500 to 3.9 pg/tube. Separation of antibody-bound and unbound 11β-A was achieved with dextran-coated charcoal.

Reliability of the 11β-A RIA was determined as follows: Accuracy was assessed by measuring 11β-A in serial dilutions (1:1, 1:2, 1:4, 1:8) of pooled serum from women (undiluted serum concentration 7.8 ng/ml). Linear regression analysis of the 11\u00bb-A concentrations measured (y) versus the expected 11β-A concentrations yielded the following equation and correlation coefficient: y = 1.17x - 0.24; r = 0.999(n = 7). The assay precision was determined at a pooled serum concentration of 2.15 ng/ml. The intraassay and interassay coefficients of variation were 11.0% (n = 7) and 12.0% (n = 18), respectively. The specificity of the 11β-A antiserum was previously characterized.6 In our assay system the cross-reactivity of the antiserum with several important steroids was androstenedione, 11.2%; testosterone, 0.04%; cortisol, 0.13%; and DHEA, 2.3%. Because our 11β-A RIA uses an efficient chromatographic step to isolate the 11B-A fraction, we expect only minimal interference by other steroids in the assay. The practical sensitivity of the 11β-A RIA was calculated to be 100 pg/ml on the basis of the lowest reliable standard curve concentration (3.9 pg per tube), an assay factor of 17.5, and an average procedural recovery of 70%.

Serum levels of DHEA, androstenedione, and tes-

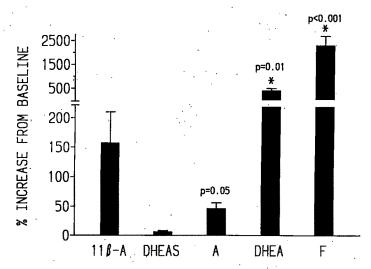


Fig. 2. Percent increase from baseline levels (mean \pm SEM) for 11 β -A, DHEAS, and rostenedione (A), DHEA, and cortisol (F) after corticotropin administration (0.25 mg intravenously) in women with hyperandrogenism (n = 16).

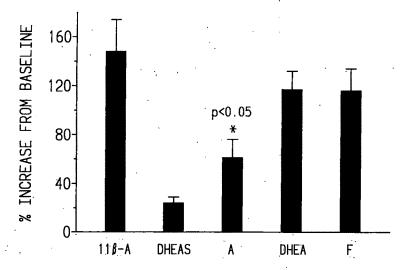


Fig. 3. Percent increase from baseline levels (mean \pm SEM) for 11 β -A, DHEAS, androstenedione (A), DHEA, and cortisol (F) after corticotropin-releasing factor stimulation (100 μ g of ovine corticotropin-releasing factor administered intravenously) in women with hyperandrogenism (n = 8).

tosterone were measured after extraction with diethyl ether and separation of steroids by Celite column partition chromatography, whereas DHEAS and cortisol were measured by direct RIA as previously described.⁷⁻¹²

Data analysis. The data from this study are reported as mean \pm SEM. Statistical tests included regression analysis by the method of least squares and the Student t test. The level of significance was 0.05.

Results

Serum levels of 11 β -A and DHEAS in normal women and women with hyperandrogenism are depicted in Fig. 1. Mean levels of both 11 β -A and DHEAS were approximately two times higher in the women with hyperandrogenism (2.69 \pm 0.16 ng/ml and 3.10 \pm 0.17

 μ g/ml) compared with the normal women (1.21 ± 0.07 ng/ml and 1.68 ± 0.16 μ g/ml). Fifty-seven percent of the subjects had elevated levels (2 SD above the mean value of the normal range) of DHEAS, that is, >2.8 μ g/ml, whereas 65% of the patients had elevations of 11β-A (>2.0 ng/ml).

Regression analysis of serum 11β -A levels versus DHEAS levels showed no correlation either in the normal women (r = 0.29); p = 0.15) or in the women with hyperandrogenism (r = 0.12; p = 0.28).

After corticotropin stimulation, 11β -A levels increased above baseline values by $157\% \pm 53\%$ (Fig. 2). This increase was significantly greater than the androstenedione and DHEAS responses, which were $46\% \pm 10\%$ (p=0.05) and $6\% \pm 2\%$ (p<0.001) above baseline values, respectively. However, the re-

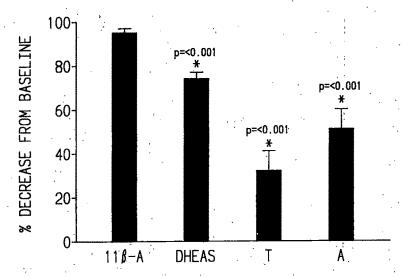


Fig. 4. Percent decrease from baseline levels (mean \pm SEM) for 11 β -A, DHEAS, testosterone (T), and androstenedione (A) after treatment with 2 mg dexamethasone daily for 3 days in women with hyperandrogenism (n = 10).

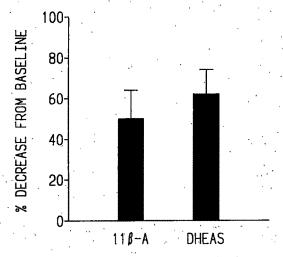


Fig. 5. Percent suppression from baseline levels (mean \pm SEM) for 11 β -A and DHEAS after treatment with 0.5 mg of dexamethasone daily for 3 months in women with hyperandrogenism (n = 7).

sponses of DHEA and cortisol were 416% \pm 80% and 2326% \pm 371% above baseline values and were significantly greater than the response of 11 β -A (p = 0.01 and p < 0.001), respectively.

With corticotropin-releasing factor, 11β -A was increased by $148\% \pm 26\%$ (Fig. 3). This was not only higher than the androstenedione response $(61\% \pm 15\%; p < 0.05)$ but also higher than the responses of DHEA and cortisol $(117\% \pm 15\%)$ and $116\% \pm 18\%$, respectively). The responses of the DHEA and cortisol, however, were not significantly different from the response of 11β -A.

Use of 2 mg of dexamethasone per day for 3 days

Table I. Sensitivity and specificity calculations for identifying dexamethasone sensitivity (adrenal gland component)

Marker	Se	nsitivity (%)	Specificity (%)	
DHEAS	. ' '	50	47	
11 β -A		83	63	

suppressed androstenedione and testosterone by $51\% \pm 9\%$ and $32\% \pm 9\%$, respectively (Fig. 4). These responses were not as great as the suppression of 11β -A (95% ± 2%; p < 0.001) or DHEAS (74% ± 3%). However, the 11β -A response was significantly greater (p < 0.001) than the response of DHEAS.

With long-term dexamethasone suppression with 0.5 mg of dexamethasone daily for 3 months, there was no difference between the 11 β -A and DHEAS responses (50% \pm 14% and 62% \pm 12%, respectively) (Fig. 5).

In both the stimulation and suppression studies, linear regression of the percentage of change of 11β-A or DHEAS versus baseline levels of the measured steroids showed no correlation (data not shown).

The sensitivity and specificity of 11β -A and DHEAS were calculated, and the values are shown in Table I. For these calculations we assumed that short-term dexamethasone suppression can be used to distinguish those hyperandrogenic women who have excess adrenal androgens. Therefore the subjects in the short-term dexamethasone study were divided into those who were dexamethasone sensitive (suppression of androstene-dione and testosterone was >60%; n=6) and those

who were dexamethasone insensitive (suppression of androstenedione and testosterone was <60%; n=18). 11β-A showed a greater sensitivity and specificity than DHEAS.

Comment

The results of this study demonstrate that 11B-A is a good marker of adrenal androgen production and may be useful in identifying those women with hyperandrogenism whose source of androgen excess is the adrenal gland. Among the group of women with mixed hyperandrogenism, DHEAS and 11B-A were elevated in 57% and 65% of the subjects, respectively. Because these percentages are not statistically different, we may conclude that in this study 11B-A is at least as valuable as DHEAS, which has been used as a specific marker. of adrenal androgen excess for some time.

On the basis of the responses of 11β-A and DHEAS to corticotropin-releasing stimulation and to short-term dexamethasone suppression, 11β-A appears to be a more sensitive marker than DHEAS for dynamic tests of adrenal gland function. One explanation for this finding may be related to the large pool of DHEAS. Additionally the pathway to the formation of 118-A may be more sensitive to stimulation than the pathway to the formation of DHEAS. 11B-A appears to be formed from androstenedione by the A5 pathway.13 However, the degree of suppression of 11β-A and DHEAS by long-term dexamethasone was similar.

Circulating levels of 11B-A and DHEAS did not correlate either in the normal women or in the women with hyperandrogenism. This finding suggests that 11β-A and DHEAS may reflect different patterns of

Although I 1β-A may be a more sensitive marker than DHEAS, there are potential disadvantages in the clinical application of 11B-A as a marker of adrenal androgen excess. One major disadvantage is that, unlike DHEAS, 116-A is subject to diurnal variation: Serum levels of 118-A are higher in the morning than at night. A second potential disadvantage of 11β-A originates from evidence that some 11β-A may be derived from the ovary. With selective venous catheterization, our preliminary data suggest that in some women the ovary may produce 11β-A.14 Another potential disadvantage of 11\beta-A is the cumbersome nature of the 11\beta-assay used in this study, in contrast to the simple and direct DHEAS assay. However, our 11β-A assay required extraction and chromatograph steps because of a lack of specificity of the 11\beta-A antiserum. A highly specific 11β-A antiserum that is used in a direct 11β-A assay is available commercially.4

Although 11β-A appears to have greater sensitivity and specificity than DHEAS (Table I), these data need to be placed in the proper perspective because the values are based on the use of the short-term dexamethasone suppression test and on a small number of subjects. We have previously shown that DHEAS is a poor predictor of this test result.15 Unfortunately there is no good criterion for establishing that a patient has adrenal androgen excess. The sensitivity of androgens to dexamethasone suppression is only one measure of dynamic adrenal androgen secretion.

Because our data suggest different secretion patterns of 11β-A and DHEAS, it is possible that both markers are required to distinguish those women with hyperandrogenism whose source of excess androgens originates from the adrenal gland.

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Effects of peritoneal macrophages from patients with endometriosis on the proliferation of endometrial carcinoma cell line ECC-1

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Endometriosis has been shown to be associated with increased number and activity of peritoneal macrophages. The peritoneal macrophage—conditioned media from 33 women with or without endometriosis were studied for their effects on an endometrial carcinoma cell line, ECC-1. The media from six of six stage III/IV cases demonstrated a mitogenic effect, which was blocked by an antibody to epidermal growth factor receptor. However, the conditioned media from seven of nine stage I/II cases and 14 of 18 normal women did not show a mitogenic effect. The difference between stage III/IV and the other two groups was significant (p < 0.01). The incorporation of tritium-thymidine was three times higher with the media from stage III/IV cases, as compared with that of controls. When purified cytokines were tested in the tritium-thymidine uptake assay, only epidermal growth factor—transforming growth factor- α was mitogenic on ECC-1, whereas tumor necrosis factor, interleukin-1, and platelet-derived growth factor had no effect. Thus peritoneal macrophages in patients with endometriosis may play an important role in the progression of endometriosis, and the noted effects could be mediated by epidermal growth factor or a related growth factor. (AM J OBSTET GYNECOL 1991;165:1842-6.)

Key words: Cytokine, epidermal growth factor, endometriosis, endometrial carcinoma cell

Endometriosis is a common disease in reproductiveaged women and is often associated with infertility. Recent studies¹⁻⁵ have shown that women with this disease have an increased number and activity of peritoneal macrophages. Growth factors (cytokines) from these macrophages or peritoneal fluid could decrease fertility by damaging gametes or embryos.^{6,7} However, whether the macrophages produce a cytokine(s) to promote the

environment by inhibiting these cells as a nonspecific inflammatory response is not clear. Stromal cell proliferation is stimulated by peritoneal fluid,⁸ so it is speculated that the development of endometriosis may be stimulated by a growth factor(s).

ectopic growth of endometrial cells or create a hostile

To determine whether a peritoneal macrophage-derived cytokine(s) from women with endometriosis has growth-promoting activity on endometrial epithelial cells, an endometrial epithelial cell line, ECG-1,9 was used for an in vitro proliferation study. To determine which cytokine has mitogenic activity on ECG-1, several commercial preparations of cytokines were compared. These preparations were tumor necrosis factor (TNF-α), interleukin-1 (IL-1), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF)—transforming growth factor-α (TGF-α).

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Material and methods

The collection and use of human tissue was approved by the Institutional Review Board of the University of Oklahoma Health Science Center. Peritoneal fluid was obtained from 15 patients with laparoscopically staged endometriosis (Revised American Fertility Society classification, 1985). Nine were stage I/II and 6 were stage III/IV. Of 9 with stage I/II, 7 were infertile; of 6 with stage III/IV, 4 were infertile. Of 18 fertile women undergoing sterilization without endometriosis, 5 had adhesions and 13 had a normal pelvis discovered at surgery; 8 were in the follicular phase and 10 were in the luteal phase of the cycle. Of 9 with stage I/II, 4 were in the follicular phase and 5 were in the luteal phase. Of 6 stage III/IV endometriosis patients, 3 were in the follicular phase and 3 were in the luteal phase.

Macrophages were isolated from peritoneal fluid by layering 1:1 diluted samples over Ficoll-Paque (Pharmacia LKB, Piscataway, N.J.) gradients and centrifuged at 400g at 18° to 20° C for 30 minutes. The macrophage fractions were washed twice with Hanks' balanced salt solution, spun at 60 to 100g for 10 minutes, and then resuspended in Dulbecco's modified Eagle's-high-glucose medium (Hazleton, Denver, Pa.) with 0.2% lactalbumin hydrolyzate (Gibco, Grand Island, N.Y.). The suspension was plated at 7.5 × 10⁵ cells per well in the 1.5 cm diameter tissue culture wells (Falcon, Los Angeles) for 1 hour. At the end of preincubation >90% of the cells were adherent. There was no difference in adherence between macrophage suspensions. The nonadherent cells were removed, and the media were then replaced with 1.5 ml of control medium, Dulbecco's modified Eagle's medium-nutrient mixture F-12 Ham with 1% fetal bovine serum (Sigma, St. Louis), and incubated as an adherent monolayer for 24 hours in 5% carbon dioxide at 37° C in a humidified incubator. Cell viability was assessed by microscopic visualization of cell morphologic types and adherence. Macrophages represented >90% of all the cells stained by Wright-Giemsa. The macrophage-conditioned media were collected, spun at 1500 rpm at room temperature for 10 minutes, and stored at -20° C until use.

A clonal continuous monolayer of the human endometrial carcinoma cell line ECC-1, which was derived from the well-differentiated estrogen-sensitive tumor EnCa-101 grown in nude mice, was used for this study because of the close similarity between the endometrial epithelial cells and ECC-1 cells. $^{9.11}$ The ECC-1 cells were plated in the 96-well tissue culture plates (Corning Glass Works, Corning, N.Y.) at a density of 1.5×10^5 per well and grown to 70% confluence in nutrient mixture F-12 Ham with 10% fetal bovine serum. The nutrient mixture F-12 Ham was supplemented with glucose 0.4 mg/ml (Sigma), insulin 0.2 U/ml (Collaborative Re-

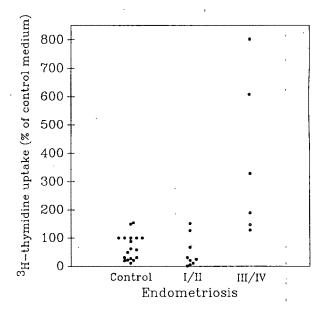


Fig. 1. Effect of macrophage-conditioned media from patients with and without endometriosis on tritium-thymidine incorporation of ECC-1 cells. Cells were harvested 24 hours after exposure to macrophage-conditioned media or control media. Difference of DNA synthesis between treatment with macrophages from patients with stage III/IV endometriosis versus other two groups is highly significant (p < 0.01).

search Inc., Bedford, Mass.), human transferrin 25 μg/ml (Sigma), cholera toxin 2.5 ng/ml (Sigma), estradiol 0.2 ng/ml (Sigma), 1% Pen/Strep mixture (Gibco), and L-glutamine 143 μg/ml (Gibco). Two days after plating, the serum-enriched media were replaced by control media (Dulbecco's modified Eagle's medium-nutrient mixture F-12 Ham, 1% fetal bovine serum) for 18 hours before addition of the macrophage-conditioned media or media supplemented with various concentrations of recombinant human TNF-α (Amgen, Thousand Oaks, Calif.), IL-1 (Accurate Chemicals, Westbury, N.Y.), PDGF (PDGF-BB, Genzyme, Boston), EGF (Genzyme), or TGF-α (Gibco). The cells were then continuously incubated for another 24 hours.

In another set of experiments ECC-I cells were preincubated with a monoclonal antihuman EGF receptor antibody (10 µg/ml, Genzyme) for I hour. Then control medium, EGF 10 ng/ml in control medium, or macropahge-conditioned medium from five patients with stage III/IV endometriosis was added to ECC-I cells, and the cells wre incubated for 24 hours. Appropriate controls were run without pretreatment with the monoclonal antibody.

Tritium-thymidine (ICN, Irvine, Calif., $0.1~\mu Ci$ per well, 10~Ci/mmol/L) was added 6 hours before the cells were harvested. The cell cultures were terminated with a semiautomatic cell harvester (Cambridge Technology, Watertown, Mass.). Incorporation of the radioactivity

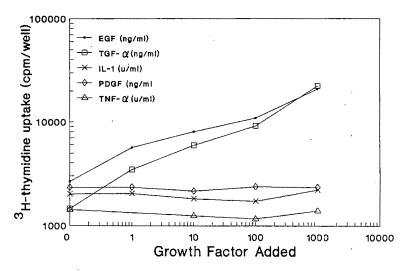


Fig. 2. Dose-dependent effect of cytokines on tritium-thymidine uptake by ECC-1 cells. EGF-TGF-α promoted tritium-thymidine uptake in a dose-dependent manner, but IL-1, PDGF, and TNF-α did not.

into cellular deoxyribonucleic acid (DNA) was measured by liquid scintillation counting.

To evaluate the effect of several cytokines on cell viability, total cell number, and cellular protein synthesis, ECC-1 cells were cultured in 1.5 cm diameter tissue culture plates at a concentration of 3×10^5 per well. Again, after the cells reached 70% confluence, they were subsequently incubated in control medium for 18 hours. The media were replaced with media supplemented with EGF (10 ng/ml), TNF- α (10 U/ml), IL-1 (10 U/ml), or PDGF (50 ng/ml) and incubated for 24 hours. Cell viability was then tested by trypan blue exclusion. Total cell numbers were counted on a hemocytometer, and protein concentrations were determined by protein assay (Bio-Rad, Richmond, Calif.).

The Wilcoxon rank-sum test was used for comparison of the mitogenic activities by macrophages from different patient groups.

Results

Macrophage-conditioned media from 6 of 6 patients with stage III/IV endometriosis demonstrated mitogenic activity to the target cell, ECC-1. Seven of 9 with stage I/II and 14 of 18 without the disease did not have the mitogenic effect (Fig. 1). The difference between stage III/IV and the other two groups was highly significant (p < 0.01). The incorporation of tritium-thymidine by the target cells was threefold higher with the medium from the stage III/IV patients as compared with that from the controls. There was no significant difference in mitogenic activity between the macrophage-conditioned media from endometriosis stage I/II and controls.

The mitogenic effect of EGF-TGF-α on target cell

DNA synthesis was dose dependent at 1 to 1000 ng/ml. In comparison, TNF- α (1 to 1000 U/ml), IL-1 (1 to 1000 U/ml), or PDGF (1 to 1000 ng/ml) showed no effect (Fig. 2). Viability of cells was >95% after the incubation period with the cytokines. Increases in total cell count and protein content were found only with the EGF (10 ng/ml) treatment, not with IL-1 (10 U/ml), TNF- α (10 U/ml), or PDGF (50 ng/ml) (data not shown).

Monoclonal anti-EGF-receptor antibody (10 μg/ml) caused >50% inhibition of the mitogenic activity of EGF (10 ng/ml, Fig. 3, B) and of macrophage-conditioned media from five of five patients with stage III/IV endometriosis (Fig. 3, C to G). The monoclonal antibody by itself had no significant effect on mitogenic activity (Fig. 3, A).

Comment

The association between endometriosis and an increase in the number and activity of peritoneal macrophages has led investigators to question whether these macrophages play an important role in the pathogenesis of the disease. Cytokines derived from peritoneal macrophages of patients with endometriosis produce different effects on various target cells. They have a proliferative effect on lymphocytes, lymphoma cells, and 3T3 fibroblasts; the mediator is thought to be IL-1, PDGF, or fibroblast growth factor.¹²⁻¹⁴ But cytokines have a cytolytic effect on L929 fibroblasts and WEHI-164 fibrosarcoma cells, which are TNF-α-sensitized target cells.^{15, 16} Peritoneal fluid from patients with endometriosis has been shown to stimulate the proliferation of human endometrial stromal cells.⁸

To study the effect of macrophage-derived cyto-

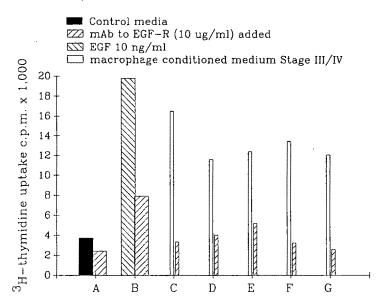


Fig. 3. Neutralization of monoclonal anti-EGF-receptor antibody (mAB to EGF-R) on ECC-1 cells. mAB to EGFR was added to control medium (A), EGF 10 ng/ml (B), or macrophage-conditioned media from patients with stage III/IV endometriosis (C to G). Antibody caused >50% inhibition of mitogenic activity of commercially obtained EGF and of macrophage-derived media from five patients with endometriosis. Antibody by itself had no significant effect on mitogenic activity (A).

kine(s) from patients with endometriosis on endometrial epithelial cells, the ECC-1 cell model was used, because an adequate number of purified epithelial cells from human endometrial tissue could not be grown. The ECC-1 cells are well-differentiated homogeneous epithelial cells that are cytokeratin positive (epithelial cell marker) and vimentin negative (stroma cell marker) by immunohistochemical methods.9 We have shown that antiendometrial antibodies from patients with endometriosis react with both endometrial glandular epithelial cells and ECC-1 cells but not with endometrial stroma cells.10 The close physiologic similarity between endometrial epithelial cells and ECC-1 cells is observed in their response to estrogen, progesterone, and interferon gamma.9, 11

Macrophage-conditioned medium from patients with stage III/IV endometriosis stimulated ECC-1 cell proliferation. Furthermore, a monoclonal antibody to EGF receptor blocked the mitogenic effect of the macrophage-conditioned medium. Because the macrophages were cultured in the absence of exogenous stimulants in vitro, these macropahges were likely to have been stimulated in the peritoneal cavity of the patients before they were studied. Therefore it is reasonable to speculate that, in the normal pelvis or in milder stages of endometriosis, the activity and secretion of macrophages might not favor the growth of ectopic endometrial epithelial cells. However, during the progression of endometriosis, as the disease becomes more severe, macrophages might receive sufficient stimuli to

be altered and reach certain levels of maturation. The macrophages might then secrete growth-stimulating substances into the peritoneal cavity, to which the ectopic cells of endometrial origin exhibit stimulated growth activity. One of the mediators might be related to EGF-TGF-α or a cytokine that reacts with the EGF receptor:

Among the tested commercial cytokines, EGF-TGFα was found to enhance proliferation, but TNF-α, IL-1, and PDGF did not. EGF and TGF-α are significantly homologous 17, 18 because they bind the same receptor. 19 Both are potent mitogens for a variety of cultured cells. In vitro, the proliferation of mouse uterine epithelial cells is enhanced by EGF but not by PDGF, fibroblast growth factor, or nerve growth factor.20 The receptor for EGF has been found in the rat uterus21 and in stromal and glandular epithelial cells of the human uterus. EGF is thought to be essential for growth of normal and neoplastic endometrium.22 Induction of transcription and secretion of TGF-α by activated human monocytes has been reported recently.23 However, it is not known whether macrophages produce EGF.

Peritoneal macrophage-derived cytokine(s) from patients with advanced stages of endometriosis and commercial EGF-TGF-α proliferated the endometrial epithelial cell line ECC-1 in vitro. These results suggested that the mediator in macrophage-conditioned media might be related to EGF-TGF-a. However, the mitogenic effect from the macrophage-conditioned medium, which was blocked by antibody to EGF receptor, could be due to other factors that work in synergy with EGF or to another factor that binds to EGF. Identification of these specific mediator(s) is currently under investigation.

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Prolonged clearance of intraperitoneal 16α -[125I]iodo-17 β -estradiol in presence of ascites

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Radioestrogens have potential as adjunct therapeutic agents against ovarian carcinomas, because selected radionuclides can deposit lethal doses of radiation to tumor cells and many ovarian carcinomas and their metastases express estrogen receptors. Because intraperitoneal administration is a possible approach, we investigated absorption from the peritoneal cavity of a radioiodoestradiol after intraperitoneal application in rats with and without ovarian tumors and ascites and compared the distribution of the radioactivity with that obtained after intravenous injection. In the absence of ascites, 70% of the intraperitoneal dose was cleared into the intestine within 2 hours after injection, indicating fast absorption from the peritoneal cavity. In the presence of ascites, clearance of intraperitoneal radioiodoestradiol was considerably slower; at 2 hours after injection, 50% of the injected dose remained in the ascites, mostly as radioiodoestradiol. Uptake of radioactivity in estrogen receptor—rich tissues, e.g., uterus, after intraperitoneal injection was high (about 20:1 over blood), regardless of the presence of ascites, but moderately lower than that observed after intravenous injection of radioiodoestradiol. (AM J OBSTET GYNECOL 1991;165:1847-53.)

Key words: Radiopharmaceutical, radioiodoestradiol, ovarian tumor, ascites, clearance

A recent report on the specific sequestration of a radiolabeled estrogen receptor ligand, 16α -[123 I]iodo- 17β -estradiol ([123 I]E $_2$), in estrogen receptor-rich tumors in man in vivo provided data for imaging of breast tumors and their metastases 1 that highlight the potential for application of radiolabeled sex steroid receptor ligands as pharmaceuticals. $^{2-6}$ In addition to being useful for imaging, iodoestrogens have been shown to cause selective cytotoxicity in estrogen receptor-rich cells in vitro. $^{7-9}$ This supports their potential for therapy. Use of radiohalogenated receptor ligands, especially those with short-range emissions (Auger electrons), in therapy is predicted not to compromise the use of established therapeutic modalities. $^{10-12}$

Ovarian carcinomas and their metastases express estrogen receptors in approximately half of the cases seen clinically, even after chemotherapy.^{18, 14} Therefore radioestrogens may offer an option as adjunct therapeu-

tic agents. Ovarian carcinoma is an intraperitoneal disease in most instances; therefore intraperitonal application of pharmaceuticals is considered an appropriate therapeutic approach.^{15, 16} We demonstrated recently that [125I]E2 given intraperitoneally is rapidly cleared from the peritoneal cavity with a half-time of approximately 30 minutes and that this route of administration results in high receptor-specific uptake in estrogen receptor-rich tissues.37 However, ovarian cancers are often accompanied by ascites. This raises the question whether the presence of ascites would affect the distribution and clearance of radiosteroids given intraperitoneally. To study this possibility, we compared organ uptake and excretion of [125I]E2 after intraperitoneal instillation into healthy female rats and into rats with ovarian tumors and ascites. We also compared the results to those obtained after intravenous injection.

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Material and methods

Radiochemical purity and chromatography. Our approach for ensuring radiochemical purity and preparation of [125 I]E₂ (specific activity about 2000 Ci/mmol; Biomedical Products, Overland Park, Kan.) was adapted from the routine procedures we used when [125 I]E₂ is used for Scatchard plot and saturation analyses to characterize estrogen receptors for clinical purposes in specimens of breast cancer. On the day of use, chromatography on Sephadex LH20 (Pharmacia Fine Chemicals, Piscataway, N.J.) in a solvent system of ben-

zene/methanol (9:1, vol/vol) was performed to repurify and establish the radiochemical purity of the [125I]E₂. Approximately 0.2 ml of the benzene/methanol solution (9:1; vol/vol; the solvent mixture in which we held the [125I]E2 after receipt from the manufacturer) that contained sufficient radiopharmaceutical for the day's experiments was loaded onto a small (1.5 ml volume) Sephadex LH20 column held in a glass insulin syringe. Solvent (benzene/methanol; 9:1; vol/vol) was then added in 0.5 ml volumes each time that the solvent completed flowing through to the level of the column top. Fractions of 0.5 ml were collected and counted on a gamma counter. The three highest counting fractions corresponding to the peak for authentic [125I]E2 (usually including the fifth milliliter) were pooled, the solvent was evaporated under a stream of nitrogen, and then a small volume (0.020 to 0.050 ml) ethanol was used to resuspend the [125I]E2 that was subsequently diluted with distilled water, physiologic salt solutions, or the recipient's serum to an appropriate volume for injection.

Animal treatments and collection of specimens. All manipulations in animals were performed while they were under ether anesthesia.

Ovarian tumors were induced in rats (U-strain, Swedish) by autologous transplantation of the ovaries into the spleen. 18 The animals used in our experiments were about 20 months old and had easily palpable intraabdominal ovarian tumors (granulosa-theca cell tumors) and ascites. Because of delays caused by extremely cold weather and animal health care issues for international shipments of animals, of 12 rats initially prepared for developing tumors in Sweden, only four survived in adequate condition to be injected with [125I]E2 in the United States. Accrual of data over multiple time points was made possible by techniques for collecting multiple samples of blood by cardiac or venipuncture. Similarly, ascites fluid was sampled in small volumes by intraperitoneal taps. After intraperitoneal injection with [125I]E2, rats were held in small cages with filter paper over the floor. The filter paper was changed at selected time intervals, and the spots of urine cut out for counting. Thus, with rats with tumors, instead of sometimes relying upon killing the animals for each time point, we made use of repetitive sampling. This was also done with tumor-free rats that received the [125I]E2 intraperitoneally to ensure data that could be compared. Within each treatment, results were consistent and compelling and allowed interpretation of biologic phenomena that could be reproduced with almost certainty by other investigators.

Intraperitoneal injections of [125 I]E₂ (20 μ Ci, 1 ml serum) were performed in 10 healthy rats (Sprague Dawley, about 270 gm body weight) without tumors and in four rats with ovarian tumors and ascites (about 380 gm body weight). Blood was drawn by cardiac puncture

at selected time intervals. To estimate the amount of radioactivity excreted by the kidneys, we sampled the urine by collection on filter paper placed at the bottoms of the animal cages. To test for differences in organ uptake of [125I]E2 after intraperitoneal and intravenous administration, we also injected [125I]E2 (25 µCi, 200 µl serum) into the tail veins of 20 healthy adult rats (Sprague Dawley, about 270 gm body weight). These rats, as well as those injected intraperitoneally, were killed at selected time points, and then organs, including the contents of the intestines, were sampled and weighed. To assess the receptor mediation of organ uptake of [125I] E2, we blocked estrogen receptor in vivo by injecting some animals subcutaneously with 500 µg diethylstilbestrol I hour before administering the radiopharmaceutical.

Tissue handling and counting of radioactivity to assess biodistribution. Radioactivity was measured in organs, organ contents, body fluids, and urine. The individual organs of the body were extirpated in their entirety, placed in appropriately sized plastic bags or small plastic tubes, and immediately snap-frozen in liquid nitrogen vapor. With respect to animals that received [125I]E2 intraperitoneally, to reduce error from contamination from intraperitoneal fluid that contained high levels [125I]E2 on the exterior of organs of the abdominal cavity, these organs were rinsed in ice cold saline solution, rapidly blotted to remove free moisture, and then the serosal surface was peeled off as much as possible. For the gut, the serosa was peeled off beginning on the mesometrial side, along the length of which a blunt probe was pushed to facilitate separation of the bowel tissues from the serosa. Organs were counted whole in a calibrated well-counter that was operated within its range for accurate counting rates. Corrections were made for background, for tissue volume absorption of radiation, and for geometry. The two latter correction calculations were established by saline solution-filled phantoms and the use of standards with known counts. Because the well y-counter had an opening of sufficient diameter, it was possible to briefly place an entire live animal in a plastic bag into the well counter for counting immediately after administering the radiopharmaceutical. If the count rate was too high, the animal was placed on a shield above the opening; the corrections for counting efficiency changes caused by differences in geometry were established with standards that contained known amounts of iodine-125 decay. This approach provided an accurate estimate of the injected dose that could be compared with the injected dose determined from counting the syringe, needle, and any tubing that were loaded with the dose for injection immediately before the injection, from which were then subtracted the counts remaining in the syringe and accessories immediately after the injection. Calculations for radio-

activity in the whole animal's blood were based on an assumption of a blood volume of 7% of body volume. as estimated from each animal's measured whole-body weight. Gastrointestinal tract contents from selected and measured segments were gently flushed three times with cold normal saline solution into vials. Usually the entire volume could be measured; however, in instances when the count rate was too high, aliquots were taken after thorough mixing to ensure homogeneous distribution of counts throughout the entire original volume. Alternatively, the geometry for counting was changed to a configuration for which the reduction in counting efficiency was known.

Blocking estrogen receptors in vivo. To assess the receptor mediation of organ uptake of [125I]E2, we blocked estrogen receptor in vivo by injecting some animals subcutaneously with 500 µg diethylstilbestrol (DES) I hour before administering the radiopharmaceutical.

Approaches for assessing metabolites. To obtain information about metabolic pathways of [125I]E2 in rats, we tested for the chemical form associated with the radioactivity in ascites and intestinal contents from rats with tumors with the following procedures. Ascites (300 μl) and contents from the upper small intestine (100 µl) were diluted in 1 ml 0.1 mol/L phosphate buffer, pH 11.0, mixed with benzene (vol/vol, 1:10), vortex blended for 10 seconds, incubated for 1 hour, and centrifuged for 30 minutes at 1200 to 1500 g for removal of particles from the aqueous phase and extraction of radiolabeled moieties soluble in organic solvents. After three extractions, we subjected 100 µl of the aqueous phase to aqueous column chromatography (Sephadex G15, Pharmacia) in 0.1 mol/L phosphate buffer, pH 11.0 (column volume, 8 ml; column diameter, 0.85 cm; flow rate, 40 ml/hour; fraction size, 2.5 ml). This aqueous phase chromatography was performed to learn how much to the radioactivity that remained in the aqueous phase after organic solvent extraction could be attributed to free 125I and how much to watersoluble conjugates of [125I]E2. Free 125I elutes soon after a single volume equal to the column void volume has passed; glucuronide and sulfate conjugates of iodoestradiol elute after passage of solvent volumes of two or more times the void volume.

To establish whether the radioactivity in the aqueous phase consisted of glucuronides and sulfates, we incubated samples of the aqueous extracts (100 µl) with glucuronidase (from Escherichia coli, Sigma Chemical Co., St. Louis; 475 U/ml in 0.1 mol/L phosphate buffer, pH 6.8) or sulfatase (from limpets, Patella vulgata, Sigma; 3.7 U/ml in cold 0.25% sodium chloride, pH 5.0) at 37° C for 2 hours. After incubation, samples were extracted twice with benzene. Radioactivity recovered in the benzene extract would represent conversion from a polar to a nonpolar moiety by enzymatic hydrolysis and thereby establish the existence of iodoestrogen conjugates.

Benzene was evaporated from the ascites extracts. The residues were resuspended in 0.5 ml benzene/methanol (9:1) and subjected to column chromatography on Sephadex LH20 (Pharmacia) column volume, 10 ml, column diameter, 0.85 cm; flow rate, 8 ml/hour; fraction size, 1 ml) in a benzene/methanol (9:1) solvent system, which yields separation of phenolic steroids.19 To learn whether organic moieties of radioactivity that were separated by column chromatography represented the original [125I]E2, we compared their elution volumes to that of [125I]E2.

In addition, these moieties were subjected to a modified dextran-coated charcoal estrogen receptor-binding assay.20 We set the conditions such that estrogen receptor (rabbit uterine cytosol that contained estrogen receptor >300 fmol/mg of soluble cytosol protein) was in excess and the sample to be analyzed was the source of putative radioligand.

Results

In healthy rats, blood concentrations of radioactivity that had been injected intraperitoneally reached a maximum of about 0.1% of the injected dose per milliliter after 30 minutes, which decreased to 0.06% ml within 4 hours after injection (Fig. 1, A). The uptake of radioactivity in the estrogen receptor-rich uterus was high and ranged from 1% to 2% of the injected dose per gram of organ weight during the first 3 hours after injection; however, the level of radioactivity in the uterus was markedly lower after 4 hours (Fig. 2, A). Uterine uptake was blocked by pretreatment with DES. The levels of radioactivity in estrogen receptor-poor organs were generally much lower than those found in the uterus, except in the liver, omentum, and thyroid, and could not be blocked with DES. The thyroid level was high after 2 and 4 hours (Fig. 2, A). The radioactivity injected into the peritoneal cavity was rapidly cleared into the intestine. The small intestine contained 70% and >80% of the injected dose at 2 and 4 hours after injection, respectively. Radioactivity in the colon, however, was low at all times up to 4 hours after intraperitoneal injection (Fig. 1, B). Excretion of radioactivity in urine was delayed compared with secretion into the intestine; it remained <2% of the injected dose during the first 4 hours after injection (Fig.

In rats with ovarian tumors (range of tumor weights, 7 to 15 gm) and ascites (range of volumes, 50 to 110 ml), clearance of [125I]E2 from the peritoneal cavity was considerably slower. At 2 hours after injection, 50% of the injected dose remained in the ascites (Fig. 1, B). More than 90% of this activity was associated with an organic compound that cochromatographed with [125] E2 in the Sephadex LH20 system (Fig. 1, D) and

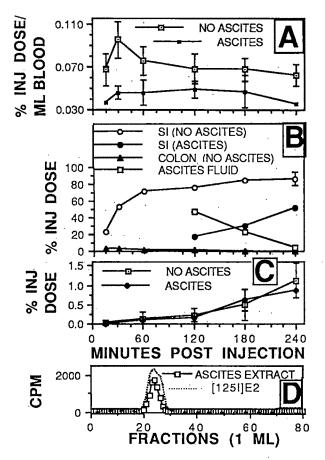


Fig. 1. Concentration of radioactivity in blood (A), fraction of radioactivity in ascites, and contents of small intestine (SI), and colon (B), and fraction excreted in urine (C) as function of time after intraperitoneal injection of [125 I]E $_2$ into rats with and without ascites. Elution patterns on Sephadex LH20 columns of [125 I]E $_2$ and of radioactivity that was extracted with benzene from ascites taken 2 hours after intraperitoneal injection (D).

bound to estrogen receptor in vitro (data not shown), consistent with its remaining [125I]E2. It took 4 hours until most of the radioactivity was cleared from the peritoneal cavity (Fig. 1, B). As in the healthy rats, low levels of radioactivity were measured in the colon content (data not shown). With the exception of a lower uptake by the omentum, the levels of radioactivity in organs, including the uterus, were similar to that observed in healthy rats. However, in contrast, uptake in the uterus, which was blocked by nonlabeled DES, remaining high at 4 hours after injection (Fig. 2, B). Ovarian tumors had a total uptake in the range of 2% to 3.5% of the injected dose, but this uptake was low on a tumor weight basis and was not altered by excess DES (Fig. 2, B). Blood levels of [125I]-label were below those seen in healthy rats and remained fairly constant for 3 hours after injection (Fig. 1, A). Approximately 20% and 50% of the injected dose of [125I]E2 was found in the small bowel at 2 and 4 hours after injection, respectively, consistent with prolonged retention of $[^{125}I]E_2$ in the ascites (Fig. 1, B).

As was determined by extraction and column chromatography (data not shown), the radioactive material in the intestinal contents did not include [125I]E₂ and contained <5% free 125I. The chemical form associated with the major portion of the intestinal radioactivity was not identified, but we found that it was not composed of [125I]-steroid conjugages that could act as substrates in our enzymatic digestions (i.e., neither glucuronides nor sulfates), because our digests did not release radioactivity that could be extracted in organic solvents (data not shown).

After injection of [125I]E₂ intravenously into healthy rats, uterine uptake was about twice that observed after intraperitoneal injection into healthy animals at the time points examined; uptake in other organs was comparable to that for intraperitoneal injection. The uterine content of radioactivity had decreased appreciably at 4 hours after intravenous injection (Fig. 3).

Comment

[125I]E2 administered intraperitoneally to healthy rats was rapidly absorbed from the peritoneal cavity, whereas absorption was delayed in rats with ascites. This difference is clearly seen in the different rates of clearance of the radioactivity into the intestine (Fig. 1). The high levels of radioactivity in the small intestine of healthy rats was found to be in the intestinal contents. Adsorption of radioactivity at the mesenteral surface of the bowel with retention in the gut lumen seems unlikely, because this would affect the entire bowel and high levels of radioactivity would then be expected also in the colon, but this did not happen. The delay in the secretion of the radioactivity into the intestine in rats with ovarian tumors can be attributed to delayed absorption from the peritoneal cavity rather than to failing liver function, because we found that a large fraction of the radioactivity was retained in the ascites (see Fig. 1, B). With liver failure, one would expect higher than normal accumulation in the liver or blood, something that did not happen.

Prolonged clearance of the radiopharmaceutical from the abdominal cavity in the presence of ascites may also occur in man. Because rats do not have sex hormone—binding globulin, this protein, which might be in ascites in man, is not responsible for the delay documented in our studies. Therefore, if the delay occurs in rats without sex hormone—binding globulin, it may be expected in humans also, who may have sex hormone—binding globulin in the ascites fluid. Possibly albumin in rat ascites fluid provides binding capability. It is important to appreciate before extending our studies on excretion patterns that only rudimentary data are available, and little is understood about differences

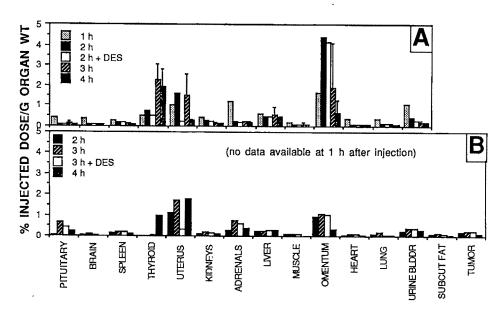


Fig. 2. Uptake of radioactivity in various organs at selected time points after intraperitoneal injection of $[^{125}I]E_2$ into rats without (A) and with (B) ascites.

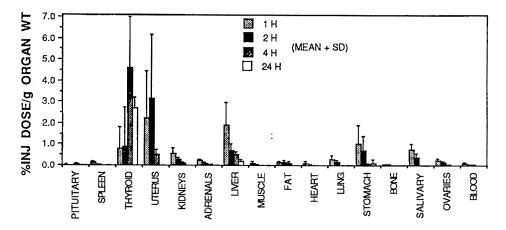


Fig. 3. Uptake of radioactivity in various organs at selected time points after intravenous injection of $[^{125}I]E_2$ in healthy rats.

in specific details of haloestrogen metabolism rat and man.

After intraperitoneal injection of [1251]E₂, high levels of radioactivity were seen in the omentum, especially in healthy rats. This uptake, which could not be blocked with DES, is most likely caused by direct absorption of the fat-soluble radioestrogen into the omentum, which consists mainly of fat. Similar results were reported after intraperitoneal injection of iodoestradiol into swine.¹⁷ The lower levels of radioactivity in the omentum in the rats with ovarian tumors is consistent with ascites affecting absorption of intraperitoneal radioestrogens.

The ovarian tumors took up radioactivity only at relatively low levels. This low uptake was predictable be-

cause these were theca granulosa cell tumors, which are known to contain and secrete steroids. The rat tumor model which we used is characterized by high levels of endogenous gonadotropins that probably result from low circulating levels of biologically active steroids, caused by the fact that the venous return from the steroid-secreting ovarian tissue transplanted to the spleen flows into the hepatic portal vein. As a result, the active steroids of the ovarian effluent are immediately conjugated in passage through the liver, and the steroidal bioactivity is lost. The high levels of gonadotropins stimulate high production and high levels of estrogens within the ovary. These estrogens occupy any estrogen receptor that might be present and block potential uptake of [1251]E₂. Most ovarian tumors seen clin-

ically, however, are not associated with a local excess of endogenous estrogens.

In the healthy animals, a major fraction of the radioactivity absorbed from the peritoneal cavity was rapidly cleared into the intestine. This finding is in accordance with earlier reports²¹ that, after intravenous administration of [125I]E₂ to rats, 50% to 70% of the radioactivity can be found in the intestinal contents within I hour, and that more than 80% of the injected dose is eventually removed in the feces. These authors reported that bile contained neither the original [125I]E₂ nor high amounts of free 125I and that enzymatic hydrolysis with glucuronidase or sulfatase did not release [125I]E₂ from bile.²¹ Their findings are supported by the results of our analyses of the radioactive material in the intestinal content.

Our data document that the onset of excretion of radioactivity into the urine is delayed considerably when compared with that into the intestine. This suggests that the radioactive metabolites of [125I]E₂ are not secreted primarily into the urine but undergo biliary clearance into the intestine. The delay in urinary excretion of radioactivity is consistent with the assumption that a major fraction of the radioactivity which finally appears in the urine was reabsorbed from the gut.

After intraperitoneal administration, [125I]E2 was available for estrogen receptor-mediated binding in receptor-rich organs, e.g., the uterus. The latter retained high levels of radioactivity for at least 3 hours. This finding is consistent with results in swine that were reported earlier.17 Intravenous injection of [125I]E2 resulted in moderately higher uterine concentrations of the radiopharmaceutical, perhaps because a significant amount of intraperitoneal iodoestradiol, after it is absorbed from the peritoneal cavity, is transported by the portal vein through the liver, which rapidly metabolizes iodoestrogens.22 Thus only a fraction of the administered radioactivity may enter the general circulation unmetabolized to be distributed throughout the body organs. Most of the estrogen receptor-mediated uptake in the uterus probably resulted from delivery through the circulatory system; radiopharmaceutical absorbed directly from the peritoneal cavity may have contributed only a fraction of the total uptake. However, the effective route of delivery may be reversed with an ovarian tumor, which metastasizes intraperitoneally as small tumor nodules or single cells, because the tumor calls in ascites take up radiohalogenated estrogens directly.23

The presence of ascites prolonged the absorption of [125I]E₂ from the peritoneal cavity but did not interfere with uptake of radioactivity in the estrogen receptor—rich uterus. Thus ascites appears to act like a depot

which could allow prolonged exposure of estrogen receptor—rich cancer cells in the abdomen to the radio-pharmaceutical. The prolonged opportunity for uptake could be expected to improve the cytotoxic potential of radiohalogenated estrogen receptor ligands against intraabdominal receptor-rich cancer cells because it would extend the time frame for formation of intranuclear receptor-ligand complex.

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C-jun and jun-B oncogene expression during placental development

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During embryogenesis, growth and differentiation occur in a sequential, predetermined order suggesting that specific genes are turned on and off in a precise and well-regulated manner. Placental development, which is characterized by massive proliferation and differentiation of multiple cell types, must be similarly regulated. Early response protooncogenes, such as c-jun and jun-B, have been associated with both proliferation and differentiation of different cell types. In this study, using Northern blot analysis, we found that c-jun and jun-B expression occurred in human placentas throughout gestation. Maximal expression of c-jun occurred in early gestation, and maximal expression of jun-B occurred in late gestation. We speculate that peak expression of c-jun in human placenta at early gestation may be related to cytotrophoblastic proliferation and that peak expression of jun-B in late gestation may be related to further terminal differentiation of trophoblastic cells. (AM J OBSTET GYNECOL 1991;165:1853-6.)

Key words: c-jun, jun-B, human placenta

During embryogenesis, growth and differentiation occur in a sequential, predetermined order, which suggests that specific genes are turned on and off in a precise and well-regulated manner. The placenta,

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which exhibits massive proliferation and differentiation of multiple cell types, must be similarly regulated. Protooncogenes may play a fundamental role in the processes of cell proliferation and differentiation. ¹⁻³ Enhanced expression of the *jun* family of protooncogenes has been associated with both proliferation and differentiation of different cell types that include fibroblasts, embryonal cells, uterine cells, and leukemic cell lines. ⁴⁻⁸ Expression of cellular protooncogenes has been described in the placenta, and evidence exists that the level of some protooncogenes varies during the course of placental development. ⁹⁻¹²

Early response protooncogenes, such as *c-jun* and *jun-B*, are believed to play a primary role in cell proliferation. We hypothesized that these genes may play

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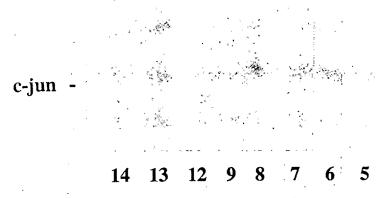


Fig. 1. Northern blot of total RNA samples isolated from placental tissues of early gestation. Expression of c-jun is noted throughout (5 to 14 weeks); maximal expression occurs at 5 and 6 weeks.

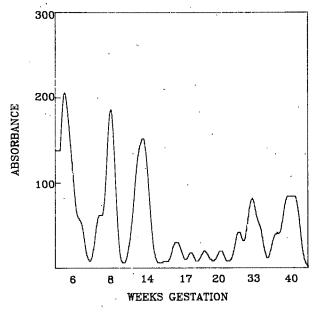


Fig. 2. Scanning densitometry graph of Northern blot of total RNA samples from placental tissues of between 6 and 40 weeks' gestation. Expression of c-jun is noted throughout gestational period shown. Maximal expression was identified in early gestation (5 to 14 weeks) by densitometric quantification.

a role in cytotrophoblastic proliferation and placental growth. We studied the temporal expression of these protooncogenes in human placentas to examine this hypothesis.

Material and methods

Collection of placental tissue. Placental tissue was obtained after curettage, spontaneous delivery, or cesarean section and was immediately placed in liquid nitrogen. Informed consent was obtained in all instances in accordance with the protocol approved by our institutional review board. Time frames of placental tissue studied were chosen to correlate with early, mid, and late gestation. Placental tissue obtained from 32 patients was used for analysis. Eight panels of total ribonucleic acid (RNA) from placental tissue of similar gestational age, ranging from 5 to 40 weeks' gestation, were studied with Northern blot analysis.

RNA isolation. Total RNA was isolated from the placental tissues according to the technique of Chomzynski and Sacchi.18 Approximately 2 gm of placental tissue was homogenized in 10 ml of extraction solution (4 mol/L guanidine thiocyanate, 25 mmol/L sodium citrate at pH 7.0, 0.5% sarcosyl, 0.1 mol/L 2-β-mercaptoethanol, 2 mol/L sodium acetate, and 10 ml phenol). The homogenate was mixed with chloroform/isoamyl alcohol (2 ml of 49:1 solution) and chilled on ice for 15 minutes. After centrifugation for 20 minutes at 10,000g at 4° C, the aqueous phase was removed and precipitated with one volume of isopropanol at -20° C for 2 hours. This was followed by a second centrifugation for 20 minutes at 10,000g and 4° C. The resultant pellet was dissolved in extraction solution (600 mm³) and precipitated with isopropanol (one volume) and sodium acetate (1/10 volume) at -20° C for approximately 2 hours. After centrifugation for 10 minutes at 4° C, the pellet was washed with 70% ethanol and vacuum dried for approximately 5 minutes. The pellet was then resuspended in diethyl pyrocarbonate-treated water. Total RNA concentration was quantitated by absorption spectrometry at 260 nm.

Northern blot analysis. RNA samples (50 and 100 μg) were resolved by electrophoresis on formaldehyde agarose gels and transferred to nitrocellulose membranes by towel blotting. The integrity and quantity of each RNA sample were ensured by staining the gel with ethidium bromide and visualizing the gel under ultraviolet transillumination. The filters were prehybridized in hybridization solution containing 10× Denhardt's solution, 0.1% sodium dodecyl sulfate, 100 μg/ml sin-

WEEKS GESTATION

jun-B -

35 33 28 26 20 17

Fig. 3. Northern blot of total RNA samples from placental tissues of mid to late gestation. Expression of jun-B is maximal at 35 and 40 weeks' gestation.

gle-stranded salmon sperm deoxyribonucleic acid, 50% deionized formamide, and a 5× solution of 3 mol/L sodium hydroxide and then hybridized in the same solution containing a phosphate 32-labeled human cjun or jun-B complementary deoxyribonucleic acid probe (1 × 106 cpm/ml) at 42° C overnight. After four washings (final wash in 1 × saline sodium citrate buffer with 0.1% sodium dodecyl sulfate at 55° C for 20 minutes), the filters were subjected to autoradiography. The position of 18S and 28S RNA was determined, and the bands that corresponded to the position of c-jun (3.2 and 2.7 kb) and jun-B (2.2 kb) were quantitated by scanning densitometry with a Hoefer GS 300 gel scanner (Hoefer Scientific Instruments, San Francisco).

Results

With Northern blot analysis using 50 and 100 µg samples of total RNA, we were able to identify c-jun and jun-B messenger RNA expression in human placentas throughout the gestational ages studied (between 5 and 40 weeks). Northern blot analysis of total RNA isolated from tissue between 6 and 40 weeks' gestation revealed c-jun expression throughout these time points. Detected levels of c-jun expression were greatest in early gestation (between 5 and 14 weeks). Further analysis of total RNA isolated from tissue samples ranging from 5 to 14 weeks' gestation revealed maximal expression at 5 and 6 weeks' gestation (Fig. 1). Fig. 2 represents the continuous scanning densitometry graph of a blot of equal amounts of total RNA isolated from placental tissue ranging from 6 to 40 weeks' gestation.

Expression of jun-B also occurred throughout gestation. Maximal levels of expression were detected later in gestation at 35 and 40 weeks (Fig. 3). Low to undetectable levels of expression were noted at early and mid gestation (between 5 and 20 weeks).

Comment

Several lines of evidence suggest a role for protooncogenes in cell proliferation and differentiation. The placenta exhibits massive proliferation and differentiation of multiple cell types during development. Knowledge of regulatory factors involved in these processes is limited, but experimental data that support a role for protooncogenes exist. Expression of protooncogenes has been described in placental tissues, and levels of expression of some protooncogenes have been shown to vary during the course of pregnancy. Expression of c-myc, for example, which has been associated with cytotrophoblastic proliferation, exhibits the highest levels in early gestation and a clear decline is seen before the end of pregnancy.14 The sis protooncogene also has been associated with cytotrophoblastic proliferation but is highly expressed throughout gestation. Expression of epidermal growth factor receptor, the cellular equivalent of the erb-B oncogene, increases throughout pregnancy and is believed to function in the maintenance of differentiation of syncytiotrophoblastic cells. 15

The expression of the different *jun* protooncogenes and the role of their gene products in cell proliferation and differentiation have been widely studied in different cell lines. To our knowledge, however, the expression of these genes has not been examined during human placental development. Because of their nuclear localization and their association with processes of cell proliferation and differentiation, the jun protooncogenes may participate in the regulation of growth and differentiation of placental tissues. Expression of jun messenger RNA has been identified in many cell lines, and an alteration of expression can be elicited when proliferation and differentiation are stimulated in these cells.16-18

Evidence exists that the regulation of different jun genes is not coordinate and that they may play distinct roles in regulation of cell growth. The relative amounts of each jun RNA vary in different cell lines and organs. 19 Although the amino acid sequence of the jun proteins is closely related, there are distinct regions of dissimilarity that have been conserved throughout evolution,19,20 which suggests that they may have distinctive biologic properties. Moreover, different levels of transcriptional activation of gene expression in response to serum growth factors have been demonstrated among the jun RNAs, which suggests that these genes may be regulated by unique controlling elements.19

The observations made in our study also suggest that the regulation of different *jun* genes is not coordinate during human placental development and that they may play distinct roles in regulation of placental growth. Although c-*jun* and *jun*-B expression was identified throughout gestation, maximal expression of these genes occurred at opposite spectra of placental development. Expression of c-*jun* messenger RNA was greatest in early gestational tissue (between 5 and 14 weeks); maximal expression occurred at 5 and 6 weeks' gestation. Conversely, *jun*-B messenger RNA expression in human placenta was greatest in late gestation at 35 and 40 weeks.

Placental growth is characterized by the proliferation and invasion of cytotrophoblastic cells; this is followed by coalescence of daugher cells in the syncytium.21 These processes predominate during early gestation. During this stage of placental development, we noted peak c-jun expression. Increased c-jun expression at this time may be functionally associated with the regulation of gene expression related to cell proliferation of cytotrophoblastic cells, as well as with the proliferation of elements of the villous mesenchymal core, including myofibroblasts and angioblastic cells. Absolute growth of the human placenta declines in late gestation; this decline begins at approximately 35 weeks, at which time cessation of cytotrophoblastic proliferation occurs.22 Maximal expression of jun-B was noted in late gestation; increased expression occurred at 35 and 40 weeks' gestation. This increase of expression at the stage of placental development during which cytotrophoblastic proliferation is absent may be associated with terminal differentiation of trophoblastic cells.

In summary, c-jun and jun-B protooncogene products have been identified as factors that regulate gene expression and that are associated with the processes of cell proliferation and differentiation. The different patterns of expression noted in different cell lines and in response to different inducing agents suggests that these genes play distinct roles in regulation of growth processes. The discordant patterns of expression of the two genes identified throughout gestation in our study by means of Northern blot analysis also suggest that these genes may regulate different gene processes during human placental development. The peak expression of c-jun in early gestation may be associated with cytotrophoblastic proliferation, and maximal expression of jun-B in the late stages of placental development may be related to terminal differentiation of trophoblastic cells. Identification of the spatial pattern of expression of these genes by means of immunohistochemical analysis or in situ hybridization will be of value in examining further the role of c-jun and jun-B in the regulation of trophoblastic cell growth and differentiation.

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New monoclonal antibodies identify the glycoprotein carrying the CA 125 epitope

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CA 125 is an antigenic determinant located on the surface of ovarian carcinoma cells and elevated in the serum of >90% of patients with carcinoma. The antigen, derived from the ovarian epithelium, has been described as a mucinlike glycoprotein >200 kd. To date little is known of the metabolic regulation or expression of this antigen in either normal or neoplastic tissues. New monoclonal antibodies that we describe here recognize both unique and similar epitopes to OC 125. These reagents may allow for a more complete definition of the structure and expression of the CA 125 complex. These antibodies recognize high-molecular weight (>200 kd) subspecies and a lower-molecular-weight (68 kd) subspecies of the antigen and identify it in the cytoplasm and the extracellular matrix of CA 125—producing cells. (AM J OBSTET GYNECOL 1991;165:1857-64.)

Key words: CA 125, OC 125, monoclonal antibodies, immunoprecipitation, immunolocalization

CA 125 was discovered by Bast et al.1,2 with a monoclonal screen for tumor-specific antigens of hybridomas derived from mouse lymphocytes immunized to an ovarian cell culture line, OVCA433. The antigen is located on the surface of ovarian tumor cells with essentially no expression in normal adult ovarian tissue.3 It is expressed on the cell surface of tumor cells in culture and on ovarian tumor lesions. Significantly, CA 125 is also found in sera of patients with ovarian adenocarcinoma. It is, however, not exclusively in blood from patients with ovarian carcinoma but is also elevated in a significant percentage of sera obtained from patients with pancreatic carcinoma (about 50% of patients) and in liver, colon, and others (22% to 32%).1.4 It is now clear that CA 125 can be detected regularly on the tumor cell surface and in the serum of patients with serous cystadenocarcinoma of the ovary (>95%). Expression of this antigen occurs less frequently in endometrial and clear cell carcinomas, and essentially no expression is detected in mucinous cystadenocarcinomas. It may be expressed by endometrial, cervical, and mucinous cystadenocarcinomas of the ovary but is detected infrequently in the blood of these patients.

The presence of CA 125 in high concentrations in the serum of patients with ovarian adenocarcinoma has significantly altered the management of such patients. Even though CA 125 is not specific for ovarian carcinoma, it does nonetheless correlate directly with disease status (progression [increase], regression [decrease], and no change [constant]).2 Almost all ovarian cancer patients receive extensive chemotherapy, and CA 125 is used by many investigators as an indicator of a disease-free state.5-7 At second-look laparotomies it has been found that focal disease of <2 cm can be present without an elevated serum CA 125 level.8.9 Nonetheless increased serum concentrations of CA 125 precede clinical diagnosis of recurrent disease from 1 to 4 months. As a result, assay of this tumor marker is becoming a standard component of the care for patients with ovarian cancer. As previously indicated, CA 125 is not an exclusive product of ovarian cancer cells. Like many other tumor markers (e.g., α-fetoprotein and human chorionic gonadotropin), it also is expressed normally early in fetal development. Kabawat et al.3 demonstrated traces in fetal tissues; the antigen was localized to the amnion and derivatives of müllerian epithelium and coelomic epithelium (including the peritoneum, pleura, and pericardium). In adult tissues the monoclonal antibody OC 125 reacted with the epithelium of the fallopian tube, endometrium, and endocervix and has also been found expressed in the apocrine sweat glands and the mammary glands. 9. 10 Hardardottir et al. 10 have further indicated the presence of antigen dur-

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ing fetal development in notochord, myocardium, pericardium, the mesonephric duct, the vitelline and allantoic ducts, and the amnion and periderm. Elevations of serum CA 125 levels in patients with endometriosis during menses and in early gestation further supports the notion that this antigen is expressed during normal growth and development. The presence of the antigen in abundance in breast milk, benign ovarian cyst fluid, and amniotic fluid further implicate CA 125 in normal cell growth and development. The presence of the antigen in abundance in breast milk, benign ovarian cyst fluid, and amniotic fluid further implicate CA 125 in normal cell growth and development.

To date little is known of the structure of this extracellular matrix molecule, and no indication of its function has been determined. The available data on the structure of CA 125 indicate that it is part of a largemolecular-weight, mucinlike glycoprotein complex that can be resolved to a 200 to 250 kd species on sodium dodecyl sulfate-acrylamide or agarose-acrylamide gels. The antigen is thought to contain a carbohydrate component, on the basis of the presence of sugar residues, buoyant density studies, and lectin-binding properties. However, the antigenic epitope recognized by OC 125 is considered to be peptidic in nature because of its sensitivity to proteases such as trypsin and V8 protease and its relative stability to glycosidases.20-22 We reasoned that understanding the structure and function of the CA 125 molecule depends both on the development of reagents to map protein domains and on cloning the CA 125 gene. Here we describe some new monoclonal antibodies that recognize both unique and common (or closely related) epitopes detected in the glycoprotein molecule that expresses the CA 125 determinant.

Material and methods

Immunization. CA 125 antigen derived from ascites fluid was partially purified (approximately 2000 U/µg protein) as previously described.17 The antigen was precipitated with alum according to the procedure of Hudson and Hay.23 The resulting precipitate was washed twice with a 15 µl volume of 0.01 mol/L phosphatebuffered saline solution with 0.15 mol/L sodium chloride, pH 7.2. The pellet was suspended in 50 µl of phosphate-buffered saline solution and maintained at 4° C until use. BALB/c mice were immunized by intraperitoneal injection of 20 µg alum-precipitated antigen in 100 µl phosphate-buffered saline solution containing 109 killed Bordetella pertussis organisms (Michigan Department of Health, Lansing, Mich.). After 4 weeks mice were challenged again by intraperitoneal injection of a 20 µg of soluble antigen in phosphate-buffered saline solution. Two weeks later the mice were challenged further with 20 µg of soluble antigen by intraperitoneal injection. Three days later spleens were harvested for fusion with mouse myeloma

Hybridomas. Fusion of immune mouse splenocyte

and 8-azo-guanine—resistant P3X63-Ag8.653 mouse myeloma cells was performed according to the procedures of de St. Groth and Scheidegger. Aliquots of 50 μ l hybridomas were transferred to each well of five 96-well tissue culture plates (Flow Labs, Costa Mesa, Calif.) already containing 200 μ l HAT medium (10⁻⁴ mol/L hypoxanthine, 1.6 \times 10⁻⁵ mol/L thymidine, and 4 \times 10⁻⁷ aminopterin) and 3600 mouse peritoneal macrophages. When hybrids developed, media were screened with a dot blot assay as described below. Positive cultures were cloned by the limiting dilution method in 96-well culture plates. After 2 to 4 weeks of growth, culture media were again screened and the positive hybrids grown up and recloned.

Screening for CA 125 antibodies. Culture media were screened with a nitrocellulose dot blot procedure. Ascites fluid with a CA 125 concentration of 22,000 U/ml was diluted 1:20 in phosphate-buffered saline solution; 100 µl was dot blotted to nitrocellulose with a dot blot apparatus (Schleicher & Schuell, Inc., Keene, N.H.). Control ascites with <20 U/ml CA 125 diluted 1:20 was also blotted to nitrocellulose. All blots were rinsed twice with phosphate-buffered saline solution and placed in blotto (5% dry milk powder in phosphatebuffered saline solution) overnight. Individual dots were cut from the blot, and 100 µl of each hybrid culture medium was added to tubes containing one control dot (low CA 125) and one test dot (high CA 125). Dots were incubated for 3 hours at room temperature, at which time 0.5 ml of blotto was added to each tube and incubated for a further 10 minutes. All tubes were then aspirated, and the dots were washed twice with 1 ml phosphate-buffered saline solution. Tubes were then incubated with a second antibody, goat/antimouse peroxidase (Biorad Corp., Richmond, Calif.) diluted 1:250; 250 µl was added to each tube. After the second antibody was incubated for 1 hour at room temperature, the tubes were aspirated; the dots were washed twice with phosphate-buffered saline solution and then exposed to horseradish peroxidase color developer as described by Biorad Corp. instructions.

Productive cultures were transferred to 1 ml culture dishes and grown out with at least two changes of media; they were then screened with a dot blot assay similar to that described above, except purified CA 125 was used as the positive test dot. Normal human serum (diluted 1:20) was used as the negative control. Screening also was carried out by saturating OC 125 beads (Centocor, Malvern, Pa.) with CA 125 antigen by exposing the beads to 1 ml of ascites fluid containing CA 125 22,000 U/ml overnight at room temperature. The beads were then washed twice with 5 ml phosphate-buffered saline solution and further incubated for 3 hours at room temperature with rabbit antimouse immunoglobulin G to saturate any OC 125 antibody not occupied by CA 125 antigen. After washing, beads were

Table I. Screening of hybridoma media*

Culture	Antibody	Dot blot	Antigen bead
4A11	M1	3+	3+
3D12	M2	3 +	3+
1C2	M3	3+	3+
1B4	M4	2+	2+
2H10	M5	3+	2+
3D6	M6	2+	2+
2E12	M7	2+	2+
1H8	M8	2+	2+
5F12	M9	1+	1+
1C11	M10	1+	1+
2G10	Mll	4+	2+
2D12	M12	4+	2+

^{*}Twelve of 24 cultures continued to produce antibodies that recognize CA 125-rich test material.

exposed to culture media for 3 hours at room temperature. The beads were washed twice with phosphate-buffered saline solution and exposed to peroxidase-coupled antibody (goat antimouse peroxidase) and incubated for 1 hour at room temperature. Beads were aspirated and exposed to peroxidase substrate for color development. Control culture medium was used to determine background levels of staining.

Competition of new antibodies for OC 125 epitope. Culture media from positive hybrids along with control culture media (from nonproductive wells) were incubated with iodine 125-OC 125 antibody in a binding assay for CA 125 antigen. OC 125 beads (Centocor kit) were exposed to 0.4 ml ascites fluid (22,000 U/ml CA 125) overnight at room temperature. Beads were washed twice with 5 ml phosphate-buffered saline solution and exposed to 100 µl of 125 I-OC 125 antibody in the presence of 100 µl of culture media from each of the productive hybrids and media from two nonproductive hybrids for a further 18 hours at room temperature. Beads were washed and counted according to kit instructions. Similar competition studies were carried out by preloading individual OC 125 beads with CA 125 antigen obtained from normal human serum, tumor serum, ascites fluid, breast milk, and amniotic fluid that contained high levels of CA 125. CA 125 beads were then exposed individually to M-2 or M-11 or to control culture media in the presence of 125 I-OC 125 as described above.

Groups of six BALB/c mice were preconditioned with peritoneal injections of 0.5 ml Pristane (Aldrich Chemical, Milwaukee) 14 days before injection with 10⁶ hybridoma cells. Ascites fluid was harvested approximately 7 to 9 days after hybridoma injection. Fluid was centrifuged to remove cells, and the supernatant containing monoclonal antibody was stored frozen (-70° C) until used.

Immunoprecipitation. Aliquots (500 μl) of normal human serum, amniotic fluid, and CA 125-positive ascites fluid were incubated with 100 μl of 1:100 dilution

Table II. Screening of hybridomas for antibodies that compete with OC 125

Monoclonal antibody	¹²⁵ I-OC 125 bound (%)	
Ml	94	
M2	28	
M3	89	
M4	98	
M5	99	
M6	94	
M7	100	
M8	86	
М9	100	
M10	92	
M11	99	
M12	100 .	
Control	98	

of each of the test monoclonal antibodies and ascites from one control mouse overnight at 4° C. Two hundred µl of rabbit antimouse immunoglobulin G Agarose beads (Biorad Corp.) diluted 1:2 were added to each tube and incubated for 4 hours at room temperature with agitation. After centrifugation, the beads were aspirated and washed twice with 1 ml buffer (5% albumin in phosphate-buffered saline solution containing 0.1% azide) and twice with 1 ml of bis-Tris buffer (20 mmol/L bis-Tris propane 7.2). The beads were then exposed to 50 µl of 2× electrophoresis sample buffer²⁵ for 3 minutes at 100° C. The beads were cooled, then pelleted at 2000 revolutions/min for 10 minutes, and the supernatant was removed for sodium dodecyl sulfate—polyacrylamide gel electrophoresis.

Electrophoresis/transblotting. Sodium dodecyl sulfate—polyacrylamide gel electrophoresis electrophoresis was carried out on 4% to 20% gradient polyacrylamide gels, according to Laemmli,25 for 4 hours at 30 mA. After electrophoresis, the gels were soaked in Trisglycine buffer (24 mmol/L Tris hydrochloride, 192 mmol/L glycine, pH 8.3) and transblotted to an Immobilon PVDF filter (Amicon Corp.) with the Tris-glycine buffer with 15% (vol/vol) methanol overnight at 30 V.

Western blotting. Immobilon filters were incubated for 30 minutes at room temperature with blotto, followed by two washes in phosphate-buffered saline solution for 10 minutes each. Blots are then exposed either to mouse ascites monoclonal antibody at a dilution of 1:100 in phosphate-buffered saline solution or to OC 125—125I antibody (Centocor kit) at a 1:10 dilution at 4° C overnight. The filters were then washed once with blotto for 10 minutes, followed by two washes in phosphate-buffered saline solution. The blot was then exposed either to x-ray film overnight if 125I—OC 125 was used or to peroxidase-coupled second antibody, (goat antimouse peroxidase) at 1:250 dilution (Biorad Corp.) if mouse monoclonal ascites was used. Second antibody incubation was carried out at room temper-

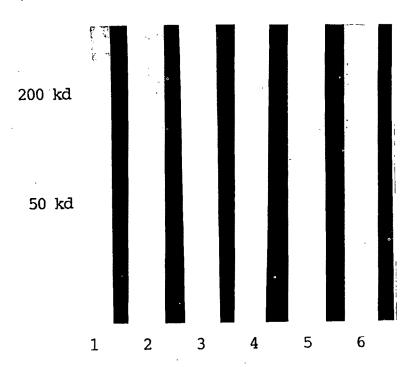


Fig. 1. Western blot of CA 125-rich (22,000 U/ml) ascites fluid. Lanes 1 to 6 were probed with monoclonal antibodies M2, 11, M6, M1, M3, and control mouse ascites, respectively.

Table III. Competition for CA 125 epitope

	¹²⁵ I-OC 125 bound (%)				
Competition medium	Ascites fluid	Amniotic fluid	Breast milk	Tumor serum	Normal human serum
Control	100	100	100	100	100
M11 Antibody	96	100	98	98	95
M2 Antibody	0.9	1.3	2.6	1.8	2.3

ature for 1 hour; filters were washed with blotto and phosphate-buffered saline solution as described above. Blots were developed with 4-chloro-I-naphthol color substrate (Biorad Corp.) as described above.

Immunohistochemistry. For immunohistochemical studies, sections (5 µm) of formalin-fixed, paraffinembedded tissues were deparaffinized and rehydrated through serial alcohol baths to water. The hydrated sections were treated with 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity, followed by exposure to normal goat serum to reduce nonspecific background staining. Primary monoclonal antibody, diluted 1:100 in phosphate-buffered saline solution, was added to sections at room temperature and incubated for 30 minutes. Linking antibody and labeling antibody were applied to tissue sections, according to kit instructions supplied by Dako (Santa Barbara, Calif.) second-antibody kits. The substrate used for localization of antibody binding was 3-amino-9ethyl-carbazole (2%). Counterstaining was achieved with Mayer's hematoxylin. Negative control slides were

prepared in identical fashion with either normal mouse serum or mouse ascites in place of the primary antibody.

Results

The initial screening process of hybrid media with low CA 125 and high CA 125 dot blot analysis yielded 24 wells with a positive dot blot test. A dot was scored positive when staining was present on the test dot (+CA 125) and negative on the control dot (low CA 125). Rescreening of these 24 expanded hybrids with both purified CA 125 and normal human serum (low CA 125) by dot blot analysis and by OC 125 bead binding yielded 12 positive hybrids of CA 125 antibody-producing hybridomas (Table I). Both dot blots and beads were graded visually on a scale of 1 to 4 for color development. Further analysis of the 12 media from the positive wells by competition analysis with 125 I-OC 125 binding to the CA 125 antigen in competition with the individual antibodies present in the positive wells indicated that media from one well described here as M2

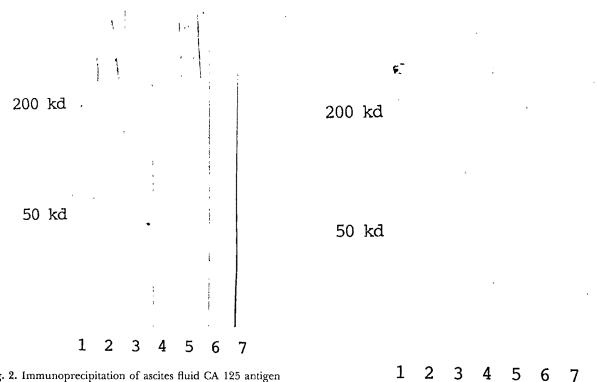


Fig. 2. Immunoprecipitation of ascites fluid CA 125 antigen with newly developed monoclonal antibodies, followed by Western blotting and probing of individual lanes with homologous monoclonal antibody. Lanes 1 to 7 were individually probed with M1, M2, M3, M6, M11, M12, and control mouse ascites.

Fig. 3. Immunoprecipitation of ascites fluid CA 125 followed by Western blotting of precipitates, as described in Fig. 2, but individual lanes were probed with 125I-OC 125.

(Table II) effectively competed for a site similar to, or the same as, the OC 125 antibody. Control media or other positive media did not compete with 125 I-OC 125 binding to CA 125. After cloning, six hybrids continued to recognize CA 125. These cloned hybridomas were then used to produce ascites in Pristane-treated BALB/c mice. When M2 antibody was produced as ascites, it competed effectively with 125 I-OC 125 whether the source of CA 125 antigen was serum or ascites obtained from tumor patients or human serum, normal amniotic fluid, or breast milk (Table III).

Western blot screening of these monoclonal antibodies indicated that antibodies designated M2 and M11 recognized high-molecular-weight antigenic determinants (>200 kd) as indicated in lanes 1 and 2 (Fig. 1). Electrophoresis patterns of nonpurified CA 125 often give a smudged appearance after blotting. This is because of the association of the antigen with mucinlike high-molecular-weight glycoproteins (lanes 1 and 2, Fig. 1). Low-intensity binding by M6 (lane 3, Fig. 1) also was detectable on the original blot but was not readily visible in Fig. 1. Immunoprecipitation of tumor ascites CA 125 antigen was carried out with monoclonal antibodies derived from the six productive hybridomas and control mouse ascites. This was followed by electrophoresis, blotting, and probing with homologous antibodies indicating that monoclonal antibodies M2 and M11 (lanes 2 and 5, respectively, Fig. 2) recognize multiple high-molecular-weight antigenic species >200 kd. Monoclonal antibody M1 recognizes a 68 kd subunit (lane 1) distinct and separate from antibodies M2 and M11. Immunoprecipitation, electrophoresis, and blotting of a second set of these antibodies probed with the heterologous 125 I-OC 125 antibody indicates that OC 125 recognizes at least one of the molecular species precipitated by both M2 and M11 (lanes 2 and 5, respectively, Fig. 3). No other immunoprecipitate, including the 68 kd recognized by M1, was readily detected with OC 125. Immunoprecipitate of antigen from CA 125-rich sources such as ascites (22,000 U/ml) and amniotic fluid (2000 U/ml) and CA 125deficient sources such as normal human serum (CA 125 <10 U/ml) further demonstrates recognition by M2 and M11 of CA 125 (Fig. 4, A and B). Immunoprecipitates of ascites from nonimmunized mice are shown in Fig. 4, C. Lane 1 in each panel, which is ascites immunoprecipitate (rich in CA 125), clearly indicates that M2 (A) recognizes high-molecular-weight antigen from this source. M2 shows only faint bands for CA 125 antigen derived from the less abundant amniotic fluid source of CA 125 (lane 2, B). Essentially no antigen can be detected in immunoprecipitates from normal hu-

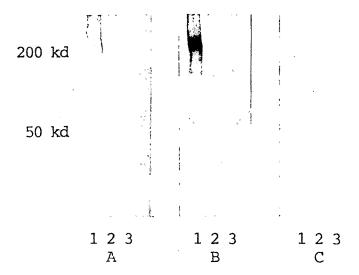


Fig. 4. Immunoprecipitation of CA 125 from ascites fluid, amniotic fluid, and normal human serum (lanes 1 to 3) with monoclonal antibody M2 (A), M11 (B), and control ascites (C) followed by Western blotting and probing with homologous antibodies.

man serum (lane 3, *B*). Similarly, probing with M11 confirms that ascites provides a rich source of CA 125 and amniotic fluid, a poorer source. M11 did not bind immunoprecipitates of normal human serum (lanes 1 to 3, *B*). Control ascites contained no antibody capable of recognizing CA 125 from all sources of the antigen (*C*). Bands at 50 kd and approximately 30 kd represent mouse immunoglobulin G heavy and light chain recognized by the goat anti-mouse second antibody.

Evaluation of antibodies by immunohistochemical localization in known CA 125-positive tissues further indicates recognition by these antibodies of antigen localized within cells also recognized by the monoclonal antibody OC 125 (Fig. 5). OC 125 has previously been shown to stain distinctly the single epithelial cellular layer of the amniotic membrane. Comparative staining of monoclonal antibodies M2 (A), M11 (B), M1 (C), and OC 125 (D) along with mouse ascites control (E) demonstrates the specific localization of these antibodies to the amnion. Distribution of antibody within the amniotic epithelial cells is more evident with M2 (A) and M1 (C), whereas extracellular or glycocalyx localization of the antibodies is evident with M11 (B) and OC 125 (D).

Comment

Expression of CA 125 during fetal development on the epithelium of müllerian duct derivatives (for example, cervix, corpus, and fallopian tube) and on derivatives of coelomic epithelium (peritoneum, pleura, and pericardium) has been documented by Kabawat et al.³ Expression of the antigen on the developmental sites is maintained through adulthood, resulting in the presence of CA 125 in all non-tumor-bearing adults.

More recently, we have described abundant levels of CA 125 in amniotic fluid, and it is now clear that both the amnion and the fetal periderm are major sources of CA 125 during gestation.17, 18 It is therefore somewhat surprising that CA 125 is a useful marker for ovarian cancer, in light of the presence in abundance of the antigen in normal tissues. Antigen produced by normal tissues may account for the low but detectable levels of CA 125 that are measurable in both men and women. The large quantities of CA 125 present in the endometrium and cervix are normally secreted to the outside without access to the circulation. In special cases, for example, in patients with endometriosis, during menses, and during embryo implantation and early gestation, CA 125 gains access to the circulation through disruption of the endothelium and results in elevated CA 125 levels. 13-15 In patients with ovarian carcinoma, access to the circulation in measurable amounts occurs relatively late in the disease process; most patients with ovarian carcinoma are diagnosed in the advanced stages (e.g., stages III and IV) when elevated levels of CA 125 might be expected. 1.4.5 As previously mentioned, at second-look laparotomy, when foci of disease are <2 cm, the CA 125 level often is not elevated or not detected in the blood.9

It is apparent that new monoclonal antibodies that have higher affinity for the CA 125 glycoprotein (1) would provide the basis for an assay system that could detect relatively low levels of serum CA 125, (2) would discriminate between CA 125 antigen synthesized by ovarian carcinoma cells and normal tissues and so would have significant importance to patient care, (3) are capable of recognizing the cell surface of tumor cells and would provide a means of radioisotope im-

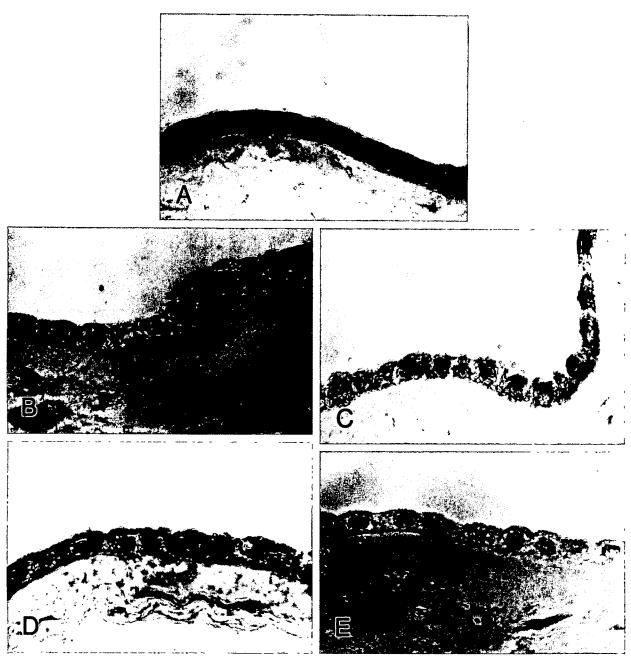


Fig. 5. Immunolocalization of CA 125 antigen in amnion epithelium with M2 (A), M11 (B), M1 (C), OC 125 (D), and control mouse ascites (E). (Original magnification ×100.)

aging or as delivery systems for therapeutic agents and would enhance diagnosis and therapy, and (4) are able to recognize individual domains of the CA 125 molecule and would simplify mapping of the biologically active sites of the CA 125 molecule, providing insight into the normal function of CA 125. Our initial efforts have yielded new monoclonal antibodies, providing the basis for initiating such studies.

These antibodies include one (M2) that competes with OC 125 for binding to the CA 125 antigen. It recognizes large-molecular-weight subspecies of CA

125 similar to OC 125 and similar to the purified subspecies described by Davis et al.22 M11, a second monoclonal antibody, does not compete for the OC 125 binding site but does immunoprecipitate multiple CA 125 subspecies similar to M2. OC 125 recognizes a major subspecies of those precipitated by M2 and M11. Even though these two antibodies recognize similar CA 125 subspecies, immunolocalization of antigen that uses the amnion as a test system indicates a significant difference in cellular distribution of determinants recognized by these two antibodies. M2 is almost entirely localized within the amniotic epithelium, relatively evenly distributed throughout the cytoplasm. In contrast, M11 is almost exclusively localized to the extracellular glycocalyx matrix and appears to recognize poorly the intracellular antigen recognized by M2. Antibody recognition of antigen localized to the extracellular matrix is of interest because drug delivery or imaging systems can best be directed with antibodies recognizing the cell surface. On the other hand, cytoplasmic recognition of the antigen is most important for antibodies that might be used to screen complementary deoxyribonucleic acid libraries where antibodies that recognize protein epitopes with no posttranslational modifications are desired. Both the M1 antibody, which recognizes a unique 68 kd subunit of the CA 125 molecule, and the M2 antibody, which recognizes high-molecular-weight subunits of the CA 125 molecule, are localized in the cytoplasm. The availability of these new antibodies will improve our ability to (1) further define the CA 125 molecule and its constituent domains, (2) screen and potentially identify the CA 125 gene(s), (3) allow more direct immunopurification of the CA 125 antigen, (4) further enhance our ability to detect and specify tumor CA 125 antigen in patient sera, and (5) direct chemotoxic agents or imaging isotopes to the surface of peritoneal tumor cells.

We thank Vincent Zurowski, Jr., Centocor, Malvern, Pa., for contributing the OC 125 antibody and CA 125 immunoradiometric assay kits.

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Specific binding sites for insulin and insulin-like growth factor I in human endometrial cancer

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Insulin and insulin-like growth factor I are known to be mitogenic and therefore may play a role in the development of endometrial cancer. We undertook this study to investigate whether human endometrial cancer tissue has receptors for these substances. Endometrial cancer tissue samples were obtained at hysterectomy from 10 women with endometrial cancer, and control endometrial tissue was collected from normal cycling women undergoing hysterectomy for nonendocrine problems. Binding studies with jodine 125-insulin and [125] insulin-like growth factor I revealed the presence of specific binding sites for insulin and insulin-like growth factor I in both normal endometrium and endometrial cancer tissue. The percent binding of [1251]insulin in the endometrial cancer tissue (mean \pm SE 2.4% \pm 0.5%/100 μg protein) was not significantly different from that in normal endometrium (3.5% \pm 1%/100 μg protein). On the contrary, the percent total binding of [125]insulin-like growth factor I in the endometrial cancer (5.3% ± 1.5%/100 μg protein) was significantly (p < 0.04) higher than that observed in normal endometrium (2.1% \pm 0.4%/100 μg protein). There was a significant positive correlation between the histologic grade of the tumor and the insulin-like growth factor I binding (r = 0.865, $\rho < 0.02$). The affinity constants for the high-affinity receptors were similar in the normal and neoplastic endometrium. These results indicate that insulin and insulin-like growth factor I may play a role in the growth and development of endometrial cancer. (AM J OBSTET GYNECOL 1991;165:1865-71.)

Key words: Insulin receptor, insulin-like growth factor I receptors, endometrial cancer

Postmenopausal women with endometrial cancer have increased serum insulin levels, and their insulin response to glucose ingestion is increased.1 Insulin and insulin-like growth factors (IGF) (specificially IGF-I) have been shown to be mitogenic, and most types of tumor cells are dependent on insulin for their proliferation.24 Insulin and IGF-I receptors are closely related to tyrosine kinase family of oncogenes.⁵ Insulin and IGF-I therefore may play a role in the growth and development of endometrial cancer. IGF-I messenger ribonucleic acid has been demonstrated in the rat uterus, and estrogens increase IGF-I messenger ribonucleic acid synthesis.6,7 It is possible that IGF-I of uterine or extrauterine origin plays a role in the growth and proliferation of the endometrial cancer cells in an endocrine, autocrine, or paracrine fashion.

We undertook this study to investigate (1) whether human endometrial cancer has binding sites for insulin

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and IGF-I, (2) if these sites are present, whether the concentrations of these receptors are increased in endometrial cancer tissue compared with normal endometrium, (3) whether the concentrations of these receptors correlate with the histologic grade of the tumor, and (4) whether there is an increase in serum IGF-I levels in women with endometrial cancer.

Material and methods

After the study was approved by the institutional review board, endometrial cancer tissue samples were obtained at hysterectomy from 10 patients with endometrial cancer. Normal endometrial tissue was obtained from women undergoing hysterectomy (in the proliferative phase of the cycle) for nonendocrine problems (cervical dysplasia, pelvic pain, pelvic relaxation). The tissue was washed in ice-cold buffer to remove any blood, quickly frozen in liquid nitrogen, and stored at -72° C until use. The day before hysterectomy fasting blood samples were obtained from all patients with cancer for measurement of insulin, glucose, and IGF-I levels. Nine of the 10 patients with cancer were obese, weighing >20% above their ideal body weights. Ideal body weights were determined from the Metropolitan Life Insurance Company tables. The ages of the patients varied from 40 to 70 years. Two of the patients were premenopausal and had a history of oligomenorrhea since menarche. The rest of the cancer patients

Patient	Age (yr)	Weight (kg)	% Ideal body weight	Fasting insulin* (þmol/L)	Fasting IGF-I† (ng/ml)
A	59	86	134	122	178
В	62	154	261	186	31
С	51	98	187	236	160
D	40	129	190	208	67
E	57	150	253	193	128
F	57	116	204	115	89
G	45	74	126	160	78
Н	51	84	137	129	138
I	70	64	104	194	131
J	54	117	192	258	72
Mean ± SE	55 ± 3	107 ± 10	179 ± 17	180 ± 16	107 ± 15

Table I. Clinical data for patients with endometrial cancer

were 1 to 20 years postmenopausal and gave a history of regular menstrual cycles before the onset of menopause. Fasting insulin levels were increased in all the patients, but fasting glucose levels were normal. Serum IGF-I levels were in the normal range. Clinical data on the endometrial cancer patients are presented in Table I.

Binding studies. To prepare endometrial membranes, we used, with minor modification, a technique developed for cartilage plasma membranes.⁸ Endometrial tissue (400 to 500 mg) was homogenized in 15 ml 0.01 mol/L imidazole, 0.25 mol/L sucrose, and 0.005 mol/L ethylenediaminetetraacetic acid, pH 7.5 (ISE buffer), at 0° C for 60 seconds at half speed with a Polytron device (Brinkmann Instruments, Westbury, N.Y.). The homogenate was centrifuged at 1000g for 15 minutes to remove nuclei, most mitochondria, and tissue fragments. The resulting supernatant was centrifuged at 35,000g for 60 minutes to provide a final pellet that was resuspended in 0.001 mol/L imidazole, pH 7.5.

Human insulin was the generous gift of Lilly Research Laboratories, Indianapolis. IGF-I and IGF-II were purchased from Bachem, Inc. (Torrance, Calif.). Insulin, IGF-I, and IGF-II were radiolabeled with iodine-125 with minor modifications of the dilute chloramine-T method of Freychet et al.9 Insulin and IGF-I binding studies were performed in 0.1 mol/L N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], 0.120 mol/L sodium chloride, 0.0012 mol/L magnesium sulfate, 0.001 mol/L ethylenediametetraacetic acid, 0.01 mol/L glucose, 0.015 mol/L sodium acetate, and 1% bovine serum albumin, pH 8.0, as previously described.10 Studies designed for Scatchard analysis of binding parameters consisted of eight different concentrations of insulin performed in triplicate. The concentrations of [125] insulin was approximately 0.05×10^{-9} mol/L, unlabeled porcine insulin was

added to achieve concentrations of 0, 0.2, 0.5, 1, 2, 10, 20, 100, and 1000×10^{-9} mol/L, and the membrane protein concentration was ≤1 mg/ml. For IGF-I binding studies unlabeled IGF-I and [125I]IGF-I were used in similar concentrations. The mixtures were incubated at 4° C for 15 to 18 hours, and the membranes were sedimented by centrifugation at 18,000g for 15 minutes and washed once in binding buffer. The results are expressed as the percentage of total radioactivity bound per 100 µg membrane protein. Protein was quantitated by the method of Lowry et al.11 Nonspecific binding was determined in the presence of at least 200fold molar excess of unlabeled insulin and IGF-I, respectively. Analysis of binding data was performed with a computer program designed to provide the best fit of a two-receptor model to the Scatchard plot. The program performs an iterative curve-stripping procedure in which a curvilinear Scatchard plot is stepwise recalculated, subtracting an increasing number of counts as a result of low-affinity specific binding until the optimal high-affinity straight line is achieved.10

Specificity studies. To evaluate the specificity of IGF-I binding, endometrial membranes were incubated in the presence of a constant amount (1×10^5 cpm) of [125 I]IGF-I, with or without increasing concentrations of unlabeled IGF-II (I to 100 nmol/L), IGF-I, or insulin (I to 1000 nmol/L). To evaluate the specificity of insulin binding, the membranes were incubated in the presence of a constant amount (1×10^5 cpm) of [125 I]insulin with or without increasing concentrations of unlabeled insulin (I to 1000 nmol/L), or IGF-II.

Affinity labeling and gel electrophoresis. Specific labeling and cross-linking with disuccinimidyl suberate and electrophoresis were performed essentially as previously described for liver membranes, 2 except separating gels were 5% polyacrylamide.

Serum IGF-I levels were measured with a kit pur-

^{*}Normal range 36 to 107 pmol/L.

[†]Normal range 36 to 233 ng/ml.

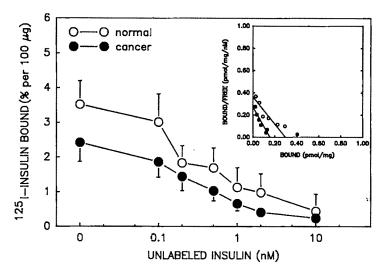


Fig. 1. Specific insulin binding (mean \pm SE) to endometrial membranes from human endometrial cancer (n = 10) and normal endometrium (n = 10). Inset, Scatchard plot of same data.

chased from Nichols Institute (San Juan Capistrano, Calif.) after extraction with acid ethanol to remove binding proteins. Insulin levels were measured by a previously described radioimmunoassay procedure. Independent Student t test was used to compare differences in specific binding between control endometrium and endometrial cancer. A p value of <0.05 was considered significant. Spearman's rank correlation was calculated between the histologic grade of the tumor and the specific binding of IGF-I and insulin to endometrial membranes.

Results

Insulin-binding studies. Specific binding sites for insulin were present in both the normal and cancerous endometrial tissue. The percent total binding of [125] Insulin in the endometrial cancer tissue (mean \pm SE 2.4% \pm 0.5%/100 µg protein, range 0.9% to 9.0%/100 µg protein) was not significantly different from that in the normal endometrium (mean ± SE $3.5\% \pm 1\%/100 \,\mu g$ protein, range 0.5% to 5.3%/100 μg protein). Fig. 1 shows the competition curves for binding of [125] insulin to endometrial membranes from normal endometrium and endometrial cancer tissue. The inset of Fig. 1 shows the mean data for Scatchard plots. The affinity constants for these high-affinity receptors were similar in the normal $(4.0 \pm 1.5 \times 10^9)$ mol/L) and neoplastic (3.0 \pm 0.6 \times 10° mol/L) endometrium. The mean receptor concentration in the neoplastic endometrial tissue (0.11 ± 0.02 pmol/mg protein) was not significantly different from that in the normal endometrial tissue (0.33 ± 0.12 pmol/mg protein). There was no correlation between the stage or grade of the tumor and the insulin-binding activity.

Specificity. Fig. 2 shows the competition curves for binding of [125I]insulin to endometrial membranes pre-

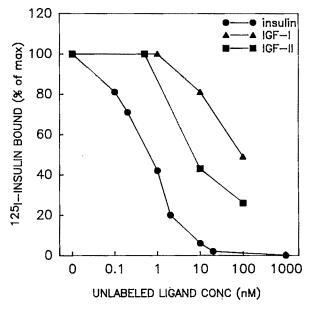


Fig. 2. Specificity of insulin binding to endometrial membranes. Each data point represents mean of triplicate determinations expressed as percent of counts bound in absence of unlabeled ligand.

pared from normal and neoplastic endometrium in the absence and presence of varying concentrations of insulin, IGF-I, and IGF-II. Increasing concentrations of unlabeled insulin resulted in dose-dependent displacement of the specific insulin binding. IGF-II was approximately tenfold and IGF-II 100-fold less potent in competing for binding. Results of these specificity studies indicate that the endometrial membranes have specific receptors for insulin.

IGF-I-binding studies. Specific binding sites for IGF-I were present in both normal and neoplastic endometrial tissue. Fig. 3 shows the competition curves

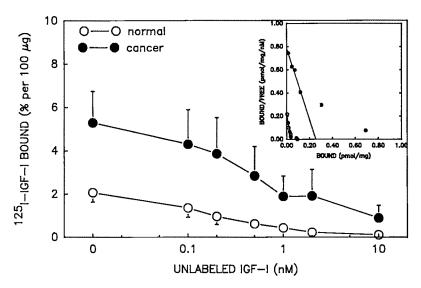


Fig. 3. Specific IGF-I binding (mean \pm SE) to endometrial membranes from human endometrial cancer (n = 10) and normal endometrium (n = 10). Inset, Scatchard plot of same data.

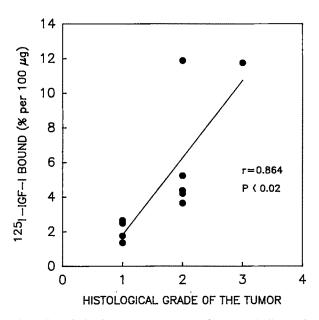


Fig. 4. Correlation between percent specific IGF-I binding and histologic grade of tumor.

for the binding of [125 I]IGF-I to endometrial membranes from normal endometrium and endometrial cancer tissue. The inset of Fig. 3 shows the mean data for Scatchard plots. The percent of total binding of IGF-I in the normal endometrial tissue was $2.1\% \pm 0.4\%/100~\mu g$ protein (range 0.5% to $4.2\%/100~\mu g$ protein). The percent of total binding of IGF-I in the endometrial cancer tissue ($5.3\% \pm 1.5\%/100~\mu g$ protein, range 1.3% to $11.9\%/100~\mu g$ protein) was significantly higher (p < 0.04) than that in normal

endometrium. The affinity constants for these high-affinity receptors were similar (8.6 \pm 1.2 \times 10° mol/L, normal endometrium; 8.1 \pm 1.8 \times 10° mol/L, endometrial cancer tissue). The mean receptor concentration was 0.05 \pm 0.03 pmol/mg membrane protein in the normal endometrium and 0.26 \pm 0.1 pmol/mg protein in the neoplastic endometrium. There was a significant correlation between IGF-I binding in the endometrial cancer tissue and the histologic grade of the tumor (r = 0.864, p < 0.02) (Fig. 4).

Specificity. The results of specificity studies on IGF-I binding are shown in Fig. 5. Increasing concentrations of unlabeled IGF-I resulted in dose-dependent displacement of specific IGF-I binding. IGF-II was five-fold less potent in its ability to compete for [125I]IGF-I binding. Insulin was able to compete for binding at higher concentrations and produced 50% displacement at 300 nmol/L concentration.

Autoradiography of dried polyacrylamide gels demonstrated specific labeling of bands of 130,000 and >300,000 molecular weight for radiolabeled insulin and IGF-I. Unlabeled insulin prevented 61% of the labeling with [125]IGF-I, whereas unlabeled IGF-I prevented 88% of the labeling with [125]insulin (Fig. 6). The 130,000-molecular-weight band is believed to be the binding subunit of type I IGF receptor. The specifically labeled larger-molecular-weight material probably represents subunits that are cross-linked together by disuccinimidyl suberate. Two bands of specific insulin binding with apparent molecular weights of 130,000 and >300,000 are similar to those observed for insulin receptors in rat liver and human placental membranes. The specific insulin receptors in rat liver and human placental membranes.

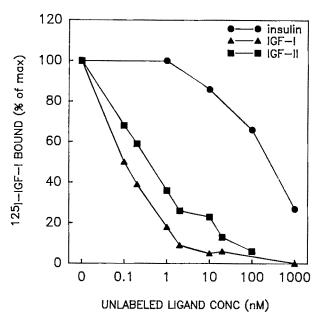


Fig. 5. Specificity of IGF-I binding to endometrial membranes. Each data point represents mean of triplicate determinations expressed as percent of counts bound in absence of unlabeled ligand.

Comment

The association of obesity and endometrial cancer is well known.14 Obesity is associated with insulin resistance and hyperinsulinemia. We have previously reported that insulin levels are increased in postmenopausal women with endometrial cancer.1 Premenopausal women with polycystic ovarian disease have hyperinsulinemia; these women are at increased risk for development of endometrial cancer at an earlier age.15 The significance of this association of hyperinsulinemia and endometrial cancer, however, is not well understood. Prolonged administration of pharmacologic doses of insulin was found to exert a carcinogenic effect in mice.16 Mammary carcinomas induced in rats were found to be dependent on insulin, and most of these tumors regressed in size or grew slower when the animals were deprived of insulin with administration of alloxan.17 Insulin stimulates growth of numerous cell lines in vitro, including breast cancer cells.18 Because the results of our study show that the neoplastic endometrial tissue contains specific high-affinity binding sites for insulin, insulin might play a role in the growth or development of endometrial cancer as well. Surrey et al. 19 observed that insulin and IGF-I stimulate proliferation of endometrial stromal cells in culture. There has been only one previous study on insulin binding in the human endometrium. Sheets et al.20 reported the presence of specific binding sites for insulin in the normal human endometrial tissue. Our results in normal endometrium are similar to those reported in this pre-

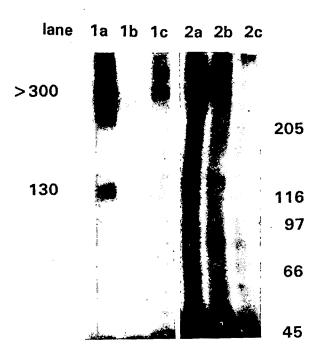


Fig. 6. Autoradiograph of 3-(trimethylsilyl)-l-propane-sulfonic acid and cross-linked affinity labeling of insulin and IGF-I receptors in endometrial membranes. Shown here is autoradiograph of endometrial membranes labeled with [125I]insulin and IGF-I in presence or absence of unlabeled ligands. Samples were reduced with mercaptoethanol and electrophoresed through polyacrylamide gels as described in Material and methods. Lane 1a contains radiolabeled insulin that is cross-linked in absence of unlabeled ligand, whereas lane 1b sample was bound in presence of 100 nmol/L unlabeled insulin and lane 1c shows labeling in presence of 100 nmol/L unlabeled IGF-I. Lane 2a shows labeling with [1251]IGF-I in absence of unlabeled ligand. Lanes 2b and 2c show labeling with [125I]IGF-I in presence of 100 nmol/L unlabeled insulin or IGF-I, respectively. Specifically labeled large-molecularweight material probably represents subunits that are crosslinked together by disuccinimidyl suberate. There is no evidence of IGF-II receptor or specifically labeled binding proteins.

vious study. We have shown for the first time that human endometrial cancer has specific binding sites for insulin. The mean insulin receptor concentration in the endometrial cancer was slightly lower than that in the normal endometrium, but the difference was not statistically significant. The decrease in insulin receptor concentration could be due to down-regulation that is a secondary effect of the elevated peripheral insulin levels. Since only a small percentage of insulin receptors are occupied at insulin concentrations that have maximal biologic effects, a decrease in receptor binding would not be expected to change the maximal effect of insulin.21 Insulin in high concentrations binds to IGF-I receptors.²² The effect of insulin therefore might be mediated either through its own receptor or by cross1870 Nagamani et al.

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over stimulation through IGF-I receptor. Our specificity data do support possible crossover action.

The other possible role of insulin in the pathogenesis of endometrial cancer is to increase the aromatization of androgens in the endometrial tissue. Tseng et al.²³ reported that the endometrium has the ability to convert androgens to estrogen by aromatization. Randolph et al.²⁴ observed that the aromatase activity in the human endometrial glands and stroma increased proportionately with increasing concentrations of insulin.²⁴ Hyperinsulinemia can therefore lead to an increase in estrogen production at the endometrial tissue level.

The human endometrial tissue has specific high-affinity binding sites for IGF-I; therefore IGF-I of intrauterine or extrauterine origin might play a role in the growth and proliferation of endometrial cancer cells. Because the peripheral IGF-I levels were not significantly different between patients with cancer and controls, IGF-I produced locally in the endometrial tissue may be acting in an autocrine or paracrine fashion. Breast cancer cells have been shown to secrete IGF-I, and 17β-estradiol stimulates IGF-I production by these cancer cells.²⁵ Endometrial cancer, like breast cancer, is an estrogen-dependent cancer. Studies by Murphy et al.^{6, 7} indicate that estrogen induces IGF-I expression in the rat uterus. Estrogen-induced proliferation of endometrial cells might be mediated through IGF-I.

The binding activity of IGF-I was significantly higher in the neoplastic endometrium than in normal endometrium. Lack of a difference in receptor affinity, as shown by the Scatchard plots, indicates that the increasing binding is primarily due to an increase in the number of receptors. The fact that there was a significant positive correlation between the histologic grade of the tumor and the IGF-I binding indicates that IGF-I may play a role in the proliferation of the tumor cells. Undifferentiated tumors, which have increased miototic activity, had an increased number of binding sites for IGF-I when compared with the number in the welldifferentiated tumors. Surrey et al.19 observed increased tritium-thymidine incorporation on addition of IGF-I to endometrial stromal cell cultures. The only previous report on IGF-I binding in endometrial cancer tissue is that of Talavera et al.26 Our results are similar to this previous report, and these authors also observed increased IGF-I binding in undifferentiated tumors. Rutanen et al.27 looked at the IGF-I receptor and IGF-I-binding protein concentrations in the normal human endometrium at different phases of the menstrual cycle. These authors observed that there is an increase in the number of IGF-I receptors and 34K IGF-I-binding protein in the late secretory phase endometrium and that the binding protein competes with membrane receptors for IGF-I binding. There was no binding protein present in the proliferative phase endometrium.27 In our study all the normal endometrial

tissue samples studied were in the proliferative phase of the cycle. Because 8 of the 10 cancer patients were postmenopausal and the two premenopausal patients had anovulatory cycles, there was probably no 34K IGF-binding protein present in the cancer tissue and all the IGF-I receptors were probably available for IGF-I action. However, this needs to be confirmed by further studies on IGF-binding proteins in endometrial cancer tissue.

In conclusion, results of our study indicate that endometrial cancer tissue has specific binding sites for both insulin and IGF-I. Because most of the women with endometrial cancer are obese and have hyperinsulinemia, insulin may play a role in the growth and development of this tumor. Since we did not find an increase in peripheral IGF-I levels in women with endometrial cancer, it is possible that IGF-I is produced locally in the tissue and acts in an autocrine or paracrine fashion.

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Increased progesterone concentrations are necessary to suppress interleukin-2-activated human mononuclear cell cytotoxicity

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Fetal trophoblast is generally resistant to lysis by cytotoxic cells. Trophoblast progesterone and estrogens may act at the choriodecidual interface, where they are present in high concentrations to provide a local, paracrine immunosuppressive effect on cellular cytotoxicity. However, interleukin activation of these cytotoxic lymphocytes enhances their ability to lyse trophoblast. Recent evidence suggests that immunoactivation occurs in certain aberrant pregnancy conditions, including preeclampsia. Preeclamptic placentas produce more progesterone in vitro than do normal placentas. To study the potential association between progesterone production and immunoactivation, we evaluated the immunomodulatory effect of progesterone on cellular cytotoxicity. Comparisons were made with the use of both normal and interleukin-2—stimulated peripheral blood mononuclear cells as effector cells in a cytotoxicity assay. Progesterone suppressed cytotoxicity in a dose-dependent manner. Interleukin-2 augmented cellular cytotoxicity, and higher concentrations of progesterone were required to attenuate this response. An additive suppression of cytotoxicity was also observed when estrone, estradiol, estriol, and progesterone were combined. We speculate that the higher placental production of progesterone seen in preeclampsia may be a trophoblast compensatory response to immunoactivated maternal effector cells. (Am J Obstet Gynecol 1991;165:1872-6.)

Key words: Progesterone, interleukin-2, cytotoxicity, preeclampsia

Local regulation of immune events at the choriodecidual interface is essential to fetal survival. Cytotoxicity by mononuclear cells is a nonspecific, primary immunodefense system that is active against foreign cells. Fetal trophoblast, however, is generally resistant to lysis by cytotoxic cells in normal gestation. Trophoblast progesterone and estrogens are present in high concentrations at the choriodecidual interface² and may exert a local, paracrine immunosuppressive effect on maternal cellular cytotoxicity. A homeostatic balance may, therefore, exist between maternal cytotoxic cells and fetal trophoblast to allow for fetal survival.

Interleukin (IL) activation of cytotoxic lymphocytes,

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however, enhances their ability to lyse trophoblast. 4.5 If a balance between maternal cytotoxic cell activity and trophoblast progesterone does exist, then a compensatory increase in placental progesterone production would be anticipated in the presence of an activated maternal immune response. Recent evidence suggests that immunoactivation occurs in certain aberrant pregnancy conditions, including preeclampsia. 6-9 An increased natural killer cell activity in patients with preeclampsia has also been observed.10 Interestingly, preeclamptic placentas produce more progesterone in vitro than do normal placentas.11 In this study we evaluated the effects of progesterone on cellular cytotoxicity with both normal and IL-2-activated lymphocytes. We hypothesized that progesterone suppresses cellular cytotoxicity and that in an immunoactivated state increased progesterone concentrations are required to achieve inhibition of cytotoxicity to levels comparable to that of the normal, unstimulated state.

Material and methods

Experimental design. In vitro cellular cytotoxicity was studied with mononuclear cells obtained from normal, nonpregnant female volunteers. Parallel experiments were performed to evaluate the immunomod-

ulatory effects of progesterone with both normal and IL-2-stimulated effector cells. To evaluate whether a combination of progesterone and estrogens could have a cumulative immunomodulatory effect, we combined estrone, estradiol, estriol, and progesterone at a 25 µM concentration each and examined this effect on cytotoxicity with IL-2-stimulated effector cells. The experimental protocol was approved by the institutional committee for the protection of human subjects.

Effector cells. Peripheral blood mononuclear cells from normal, nonpregnant female volunteers were used as effector cells. Approximately 20 ml of peripheral venous blood was obtained from each volunteer and processed within 1 hour of collection. Blood samples were sedimented with 10% (vol/vol) of 3% dextran (Sigma Chemical Co., St. Louis). The leukocyte-rich plasma was then subjected to Ficoll-Hypaque (Pharmacia Laboratories, Inc., Piscataway, N.J.) buoyantdensity centrifugation. The leukocyte layer was aspirated from the plasma-Ficoll interface. These cells were washed three times in Hanks' balanced salt solution (Grand Island Biological Co. [Gibco], Grand Island, N.Y.) and resuspended to a concentration of 3×10^6 cells per milliliter in minimum essential media (Hazelton Research Products, Denver, Pa.) supplemented with 10% inactivated fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), penicillin (50 U/ml), streptomycin (50 µg/ml), and L-glutamine (200 mmol/L). The cell suspension was then divided into two aliquots. One served as a control, and the other was resuspended and preincubated for 2 hours in the supplemented minimum essential media described, to which was added 100 U/ml of recombinant IL-2 (R and D Systems, Minneapolis). Further dilutions of both effector cell suspensions were performed to prepare effector-to-target cell ratios of 60:1, 30:1, and 15:1. Effector cell viability was determined by means of trypan blue exclusion at the time of cell suspension preparation and also after the experiment.

Cytotoxicity assay. Totals of 100 µl of effector cells (either normal or IL-2 stimulated), 50 µl of target cells, and 50 µl of reagent (supplemented minimum essential medium for controls; progesterone alone or in combination with estrone, estradiol, and estriol for experimental groups) were added to each well in U-bottom, 96-well culture plates (Corning Glass Works, Corning, N.Y.). Steroids were obtained from Sigma. All samples were run in triplicate to reduce intraassay variability. Spontaneous chromium release from the target cells was measured in triplicate wells containing only target cells and supplemented minimum essential medium. The covered plates were incubated as described for 18 hours. The amount of chromium released from the target cells was used as a quantitative indicator of cellular cytotoxicity. This was determined by aspirating 100 µl from the top of each well without disturbing the pellet and transferring it to a glass tube. To each remaining pellet, 100 µl of 1 N sodium hydroxide was added to lyse the cells. This total remaining volume was aspirated into a separate glass tube. All samples were assayed for radioactivity in a γ-counter (model 5010, Packard Cobra, Downer's Grove, Ill.) for 1 minute. Chromium release was calculated as follows:

% 51 Cr Release =
$$\frac{2A}{A + B} \times 100$$

where A is the counts per minute in the top 100 ml of supernatant and B is the counts per minute in the remaining pellet. Cellular cytotoxicity was calculated as a percentage:

$$\frac{\%}{100}$$
 for Release [(targets + effectors) - (targets only)] $\times 100$

Statistical analysis. Statistical analysis was done by analysis of variance. Individual groups were compared with the Scheffé post hoc test. A probability of p < 0.05was chosen to represent statistical significance.

Results

Effector cell viability was confirmed at >98% with trypan blue exclusion both before and after the experiments. A rise in cytotoxicity corresponding to increasing the effector/target ratios in the control wells suggested that the experimental method was valid. To optimize cellular cytotoxic effects all experimental wells were then evaluated with an effector-to-target cell ratio of 60:1. The data shown represent the mean percent cytotoxicity ± SE.

Progesterone suppressed cytotoxicity of unstimulated effector cells in a dose-dependent manner: 16.9 ± 1.5 (control), 11.5 ± 1.7 (50 μ mol/L), $7.2 \pm$ 0.9 (100 μ mol/L), 6.4 \pm 1.3 (200 μ mol/L), and 0.7 ± 0.7 (400 µmol/L). The addition of IL-2 produced a marked increase in the baseline percent cytotoxicity: 16.9 ± 1.5 (control) and 53.5 ± 3.7 (IL-2) stimulated): these values represent a threefold augmentation of cytotoxic activity. Progesterone also inhibited cytotoxicity in the IL-2-stimulated group in a dose-dependent manner: 53.5 ± 3.7 (control), $39.6 \pm 5.0 \ (50 \ \mu mol/L), \ 23.0 \pm 3.6 \ (100 \ \mu mol/L),$ 10.7 ± 2.4 (200 μ mol/L), and 2.8 ± 1.7 (400 μ mol/L) (Fig. 1). The combination of progesterone, estrone, estradiol, and estriol, each at a concentration of 25 µmol/L produced an additive suppression of cytotox-

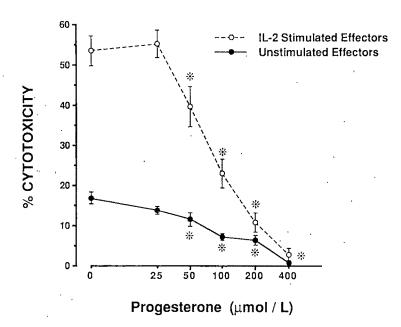


Fig. 1. Effects of progesterone on cellular cytotoxicity with unstimulated (n = 4) and IL-2-stimulated (n = 4) mononuclear effector cells. Results are expressed as mean percent cytotoxicity \pm SE. Effector/target cell ratio = 60:1. Asterisk, p < 0.05 (compared with wells not containing progesterone).

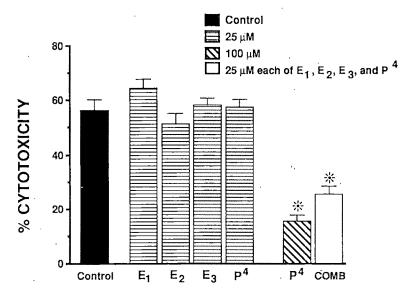


Fig. 2. Synergistic suppression of IL-2-stimulated cellular cytotoxicity resulting from combination (n=6) of estrone (E_1) , estradiol (E_2) , estriol (E_3) , and progesterone (P^4) each at 25 μ mol/L concentrations. Effector cells were IL-2 stimulated. Results are expressed as mean percent cytotoxicity \pm SE. Effector/target cell ratio = 60:1. Asterisk, p < 0.05 (compared with wells not containing progesterone).

icity in the IL-2-stimulated group: 56.5 ± 3.9 (control) and 25.6 ± 3.1 (combination). The degree of immunosuppression was equivalent to that of $100 \mu mol/L$ progesterone (Fig. 2).

To evaluate whether progesterone's ability to suppress cytotoxicity was due to stabilization of the target cell membranes, spontaneous chromium release was measured from target cells incubated with increasing concentrations of progesterone but in the absence of effector cells. No difference was observed in the spontaneous chromium release with increasing progesterone concentrations, suggesting that the observed progesterone effect was not due to target cell membrane stabilization.

Comment

Our results demonstrate that IL-2 stimulation of effector cells results in enhanced cytotoxicity against a susceptible target cell population. Progesterone suppressed cytotoxicity in a dose-dependent manner in both the unstimulated and IL-2-stimulated groups. Of particular interest is the finding that for any given percent cytotoxicity a greater progesterone concentration was required to suppress cytotoxic activity in the IL-2stimulated group as compared with the unstimulated effector cells. For example, roughly 17% cytotoxicity is the baseline value, in the absence of progesterone, for cytotoxicity in the unstimulated group. To achieve this same percent cytotoxicity in the IL-2-stimulated group, approximately 150 µmol/L progesterone was required. These findings supported our hypothesis that higher steroidal concentrations are necessary to suppress immunoactivated effector cells.

Since the normal hormonal milieu at the maternalfetal interface contains multiple steroids, we investigated the effect of a combination of steroids on cytotoxicity by immunoactivated cells. We have previously shown that the interaction of multiple sex steroids acts synergistically to inhibit cytotoxic cell activity with unstimulated effector cells.3 This concept was confirmed in this study by demonstrating an additive steroidal suppression of IL-2-stimulated mononuclear cell cytotoxicity.

During normal gestation fetal trophoblast are generally resistant to lysis by cytotoxic cells.1 Progesterone has been observed to inhibit in vitro cytotoxic cell activity.3, 12 A balance between maternal cellular cytotoxicity and trophoblast progesterone production may exist at the maternal-fetal interface, thereby allowing for fetal survival. Immunoactivation of cytotoxic cells by IL-2 augments their ability to lyse trophoblast target cells.4,5

The mechanism of progesterone immunosuppression is unclear. Previous reports suggest that a classic progesterone receptor-mediated process may not be involved.13 We have demonstrated that progesterone's suppression of cytotoxicity is not due to target cell membrane stabilization. In our study the effector cells used were a heterogeneous population of mononuclear cells. Because cytotoxic cells may be driven by macrophage IL-1 and because progesterone in vitro suppresses monocyte IL-1 production,14,15 one could speculate that progesterone inhibits cytotoxicity indirectly by decreasing macrophage IL-1 production and subsequent activation of cytotoxic cells.

Evidence suggests that preeclampsia may be an immunoactivated state. Musci et al.6 demonstrated that mitogenic activity was significantly increased in prepartum, preeclamptic sera compared with controls. Elevated sera levels of IL-2 and interferon gamma were recently observed in patients with preeclampsia.17 Rodgers et al.8 noted that serum from women with preeclampsia is cytotoxic to endothelial cells in vitro, and both immunoglobulin G and immunoglobulin M antiendothelial cell antibodies have been reported in the sera of women with severe preeclampsia.9 With specific attention to cytotoxic immune mechanisms, Toder et al.10 demonstrated an increase in natural killer cell activity in women with preeclampsia, although contrary results have been reported.16 If the presumed lymphocyte-trophoblast immune balance at the maternal-fetal interface is to be maintained, then an increase in trophoblast production of progesterone would be expected in the face of maternal cytotoxic cell activation. Such a compensatory trophoblast response would be of evolutionary importance to ensure placentation in the presence of adverse host conditions. Preeclampsia is seemingly associated with maternal immune activation; therefore it is interesting to note coincidentally that preeclampsia placentas produce more progesterone in vitro than do normal placentas.11

In conclusion, we have demonstrated that progesterone inhibits cytotoxicity from both normal and IL-2-stimulated mononuclear cells. In the immunoactivated state significantly higher concentrations of progesterone are required to achieve suppression of cytotoxicity, to levels comparable to those of the normal, unstimulated state. Estrogens and progesterone can act synergistically when combined to suppress cytotoxicity, thereby requiring lower concentrations of each individual steroid. We speculate that the increased placental production of progesterone seen in preeclampsia may represent a trophoblast compensatory response to maternal immune activation.

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Sister chromatid exchange frequency in directly prepared cytotrophoblasts: Demonstration of in vivo deoxyribonucleic acid damage in pregnant women who smoke cigarettes

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Assessing frequency of sister chromatid exchange is a sensitive method of monitoring exposure to clastogens, mutagens, and other substances that induce deoxyribonucleic acid damage. Aware that cigarette smcke is associated with increased sister chromatid exchange in many cell types, we sought to determine whether an in vivo effect of cigarette smoke could be demonstrated by study of sister chromatid exchange in chorionic villus cells. Directly prepared cytotrophoblasts and cultured mesenchymal core cells were analyzed. Mean sister chromatid exchange frequency in cytotrophoblasts from smoking subjects (8.87 sister chromatid exchanges per cell) was significantly greater than in nonsmoking subjects (5.81 sister chromatid exchanges per cell; p < 0.001); however, no significant difference in cultured mesenchymai core cells was found. Our results demonstrate that maternal exposure to cigarette smoke results in direct placental deoxyribonucleic damage, which in turn could explain deleterious effects of smoking on pregnancy. Increased sister chromatid exchange frequency was observed only in directly prepared cytotrophoblasts, showing the necessity of using this cell type to evaluate the effects of clastogens on placentas. (Am J OBSTET GYNECOL 1991;165:1877-80.)

Key words: Chorionic villi, cigarette smoke, sister chromatid exchange, prenatal diagnosis

Assessing frequency of sister chromatid exchange is a method of monitoring exposure to clastogens, mutagens, and other substances that induce deoxyribonucleic (DNA) damage.1,2 Although cigarette smoke has been associated with increased sister chromatid exchange frequency in many cell types,3,4 to our knowledge there have been no previous attempts to evaluate the in vivo effect of cigarette smoke on chorionic villi. We thus sought to compare sister chromatid exchange frequencies in directly prepared cytotrophoblasts and cultured mesenchymal core cells from chorionic villi obtained from both smoking and nonsmoking women to assess placental DNA damage resulting from cigarette smoke. We have previously shown that baseline sister chromatid exchange rates differ between directly prepared cytotrophoblasts and cultured mesenchymal core cells derived from the same villi5; accordingly, both cell types were investigated in our study.

Material and methods

Chorionic villi were obtained in the first trimester from women undergoing either chorionic villus sampling or pregnancy termination. A detailed cigarette smoking history was obtained before the procedure, and a peripheral blood sample (5 ml) was obtained for thiocyanate assay to verify smoking status.6 After aneuploid complements were excluded, all other chorionic villus specimens were coded so that laboratory personnel were unaware of medical or smoking history.

Both directly prepared cytotrophoblasts and cultured mesenchymal core cells were then analyzed as previously described.5 Briefly, villi were cleaned and incubated in complete medium (Alpha-minimal essential medium) supplemented with 15% Chang medium, 15% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 1% L-glutamine) at 37° C for 15 hours. Thereafter, intact chorionic villi were exposed to 5-bromo-2'-deoxyuridine for 72 hours at a final concentration of 30 µg/ml.

Relevant to this study is that in our cytogenetic method cytotrophoblasts and mesenchymal core cells are each are derived from the same villi rather than from different portions of a given specimen.7 To

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Table I. Results

Case No.	Smoking status	Direct (sister chromatid exchanges per cell, mean)	Culture (sister chromatid exchanges per cell, mean)		
. 1	Nonsmoker	5.2	10.1		
2 .	Smoker	9.1	10.5		
3	Nonsmoker	7.0	10.4		
. 4	Nonsmoker	6.7	10.2		
. 5	Smoker	7.6	10.0		
. 6	Nonsmoker	4.8	- 10.0		
7	Nonsmoker	6.7	9.4		
' 8	Nonsmoker	5.5	10.0		
. 9	Smoker	7.9	10.0		
10	Smoker	10.7	11.0		
· 11	Nonsmoker	4.9	9.6		
. 12	Nonsmoker	5.2	10.8		
13	Nonsmoker	5.7	10.7		
14	Nonsmoker	5.4	10.2		
15	Nonsmoker	5.7	9.5		
16	Smoker	9.5	10.4		
17	Nonsmoker	5.5	10.6		
. 18	Smoker	8.4	11.4		
. 19	Nonsmoker	5.3	10.7		
20	Smoker	8.9	10.2		
21	Nonsmoker	7.8	10.3		

achieve this, chorionic villi were first treated with trypsin—ethylenediaminetetraacetic acid to facilitate separating cytotrophoblasts from the mesenchymal core. Cytotrophoblasts were then detached by vigorously flushing villi in a Pasteur pipette. Remaining portions of villi represent the mesenchymal core, which were used to initiate long-term cultures. Cytotrophoblasts were then harvested and chromosome preparations were made after treatment with colcemid for 30 minutes.

Mesenchymal core cells derived from the same villi were allowed to remain in culture for approximately 12 days. These cells were then subcultured and 5 to 6 hours later exposed to 5-bromo-2'-deoxyuridine (final concentration 30 µg/ml) for 48 hours. Harvesting and chromosome preparations were performed in a manner similar to that used for cytotrophoblasts. Differential staining for sister chromatid exchange in cytotrophoblasts and mesenchymal core cells was according to the method of Perry and Wolff.⁸

Results

A total of 24 specimens were submitted for sister chromatid exchange analysis; 10 were obtained from patients undergoing chorionic villus sampling (10.8 to 12.7 weeks' gestation) and 14 from patients undergoing pregnancy termination (8.1 to 13.0 weeks' gestation). Three specimens, all obtained from patients undergoing pregnancy termination, were not analyzed because of their aneuploid chromosome complements. Mean sister chromatid exchange frequencies were ob-

tained from directly prepared cytotrophoblasts and cultured mesenchymal core cells for each of the 21 analyzed cases. Twenty cells were analyzed from each preparation.

Among the 21 cases analyzed, seven samples were obtained from smokers and 14 from nonsmokers. Each of the seven smoking women used at least 10 cigarettes daily; the 14 nonsmokers reported using no tobacco products (including smokeless products) for at least 6 months before chorionic villus sampling or pregnancy termination. All smokers had thiocyanate levels >55 mg/L (mean \pm SD 74 \pm 12.4 mg/L), whereas all nonsmokers had levels <30 mg/L (mean \pm SD 19.6 \pm 6.7 mg/L).

Mean sister chromatid exchange frequency for directly prepared cytotrophoblasts in smokers was 8.87 ± 1.05 (mean ± SD sister chromatid exchanges per cell), significantly greater than the frequency for directly prepared cytotrophoblasts obtained from nonsmokers $(5.81 \pm 0.89; p < 0.001, two-sample t test)$ (Table I). By contrast, sister chromatid exchange frequencies of cultured mesenchymal core cells in smokers and nonsmokers were not significantly different, 10.5 ± 0.38 and 10.18 \pm 0.45, respectively (p = 0.122; two-sample t test). In addition, sister chromatid exchange frequencies in cytotrophoblasts and mesenchymal core cells were significantly different, thus confirming results of our previous study.5 This difference was observed irrespective of whether chorionic villi were obtained from smokers (p < 0.01; two-sample t test) or nonsmokers (p < 0.001; two-sample t test).

Comment

Increased frequency of sister chromatid exchange has been shown to be a sensitive indicator of DNA damage in a variety of tissues, 9, 10 evaluating the clastogenic and mutagenic effects of drugs and environmental toxins.11 Increased frequency is also associated with certain chromosome breakage syndromes (e.g., Bloom syndrome, xeroderma pigmentosum).12,13 Sister chromatid exchange studies in humans have, in general, been limited to analysis of lymphocytes,4.9,10,14 although other tissues have been studied. 15, 16 Lukusa et al. 15 found no significant intraindividual or interindividual variability in sister chromatid exchange frequencies of human lymphocytes, whereas their similar analysis of human fibroblasts revealed significant interindividual variability. Thus analysis of fibroblasts probably should be avoided for comparisons of sister chromatid exchange frequences between individuals. Moreover, fibroblast analysis precludes in vivo assessment, for in vitro culturing is required in this tissue. For this reason, we investigated chorionic villi, a tissue amenable to direct and culture cytogenetic analyses. Our group⁵ first demonstrated that background frequencies differ between directly prepared cytotrophoblasts and cultured mesenchymal core cells. We were thus prepared to analyze frequency of both cell types after in vivo exposure to cigarette smoke.

Our current data demonstrate a significant increase in sister chromatid exchange frequency in directly prepared cytotrophoblasts obtained from women who smoke at least half of a pack of cigarettes per day. This is consistent with previous studies of the effect of smoking on frequency in maternal and fetal lymphocytes3,4; however, our results are indicative of direct placental DNA damage, which in turn could explain the deleterious effects of smoking on pregnancy, such as intrauterine growth retardation17 and late fetal and early neonatal death.18 That the increase in sister chromatid exchange frequency was found solely in cytotrophoblasts further verifies the in vivo effect on the placenta. This finding shows the desirability of using directly prepared cytotrophoblasts to evaluate the potentially deleterious effects of toxins on placentas.

Ability to analyze sister chromatid exchange frequencies in chorionic villus cells could prove useful for first-trimester prenatal diagnosis of chromosome breakage syndromes (e.g., Bloom syndrome, xeroderma pigmentosum), supplanting current techniques for prenatal diagnosis that require either amniocentesis or fetal blood sampling in the second or third trimester. 19, 20 Moreover, sister chromatid exchange analysis of placental tissue also could allow first-trimester evaluation of clastogens or mutagens that potentially affect fetal growth and development. Although our results indicate that sister chromatid exchange frequencies of cultured mesenchymal core cells do not reflect exposure to toxins such as cigarette smoke, further studies are needed to assess the applicability of both chorionic villus cell types for prenatal diagnosis of chromosome breakage syndromes and exposure to mutagens or clas-

We conclude that assessing sister chromatid exchange frequencies in directly prepared cytotrophoblasts is a sensitive indication of in vivo maternal-fetal exposure to cigarette smoke. Our findings further suggest that deleterious effects of smoking could be mediated by direct placental DNA damage.

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Editors' note

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the JOURNAL should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Cellular localization of müllerian inhibiting substance messenger ribonucleic acid during human ovarian follicular development

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Müllerian inhibiting substance is expressed in the human reproductive system and has been associated with oocyte meiotic arrest. In situ hybridization was used to selectively localize ovarian cells containing high levels of müllerian inhibiting substance messenger ribonucleic acid, a müllerian inhibiting substance precursor, during different stages of human follicular development. Müllerian inhibiting substance transcript was noted in the granulosa cells of primordial, primary, and antral follicles. Surprisingly, transcript was also identified within the cytoplasm of oocytes and throughout the ovarian stroma. Controls included sense oligoprobe, positive and negative tissue controls, and treatments minus the detection antibody. Localization of transcript within the cytoplasm demonstrates that active transcription of müllerian inhibiting substance messenger ribonucleic acid occurs within both fetal and adult human female gonads. The presence of müllerian inhibiting substance messenger ribonucleic acid within oocyte cytoplasm could implicate an autocrine role for müllerian inhibiting substance-derived peptides in the establishment of oocyte competence. (AM J OBSTET GYNECOL 1991;165:1881-6.)

Key words: Müllerian inhibiting substance (MIS), oocyte competence, in situ hybridization, messenger ribonucleic acid (mRNA), digoxigenin

Müllerian inhibiting substance is a glycoprotein associated with the regression of müllerian primordia in male embryos1 and with the sex reversal of freemartin gonads.24 Although this protein was initially thought to be expressed only in male fetuses, abundant evidence now exists suggesting that müllerian inhibiting substance plays some role in normal ovarian physiologic composition.5,6 The bioactive protein7 and messenger ribonucleic acid (mRNA) transcript8 for müllerian inhibiting substance have been identified in adult mammalian ovaries. Moreover, a sensitive radioimmunoassay has detected low levels of müllerian inhibiting substance in the cord blood of human female newborns with higher amounts detected in the sera of girls in later childhood, adolescence, and adulthood.9 The gene coding for müllerian inhibiting substance has been cloned, 10-12 sequenced, 10-12 and mapped to an autosome, chromosome 19 subband p13.2-p13.3.13

Takahashi et al.14 postulated that müllerian inhibiting

substance may serve as a meiotic inhibitor after demonstrating the absence of meiosis reactivation in mature rat oocytes exposed to the natural bovine hormone. Subsequently, others showed that human recombinant müllerian inhibiting substance can also inhibit meiosis in rat oocytes in vitro and that the addition of epidermal growth factor, an apparent müllerian inhibiting substance antagonist,15,16 can reverse this inhibition.17 Although the mechanism of this inhibition remains open to speculation, it is clear that further studies designed to uncover the full nature of müllerian inhibiting substance gene expression in the ovary are necessary. We recently elected to use in situ hybridization to selectively localize human ovarian cells containing high levels of müllerian inhibiting substance mRNA, a müllerian inhibiting substance precursor. This technology allows for the detection of small quantities of specific ribonucleic acid transcript within the cell of origin while maintaining histologic integrity of the tissues. 18 Because folliculogenesis is initiated during fetal life in the human,19 we studied ovaries at different stages of fetal and postnatal development.

Material and methods

Specimens. Formalin-fixed, paraffin-embedded archival ovaries from a 21-week fetus, a 40-week fetus, a 5-month-old infant, and a 26-year-old woman were provided by the Pathology Department at the Medical College of Georgia after clearance by the institutional

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Fig. 1. Section of ovary from 5-month-old infant. Probe concentration was 30 ng per section. All blue staining represents reaction product. A, Müllerian inhibiting substance antisense probe. B, Matched müllerian inhibiting substance sense probe. C, Same section hybridized to müllerian inhibiting substance antisense probe followed by omission of antidigoxigenin antibody. (Nuclear fast red. Original magnification $\times 1$.)

Human Assurance Committee. All tissue blocks evaluated appeared morphologically normal, had no evidence of autolysis, and exhibited little or no fixation artifact on initial histologic screening. All tissue blocks were taken from autopsies done after Jan. 1, 1989. By departmental protocol all specimens were fixed in formalin within 6 hours of receipt. Additionally, a fresh ovarian specimen was obtained, formalin fixed, paraffin embedded, and included in our observations. This specimen was obtained from a 5-month-old baby who died of secondary complications of bronchopulmonary dysplasia.

Briefly, 5 μ m tissue sections were mounted on slides pretreated with a 2% solution of 3-aminopropyltriethoxysilane (silane) in acetone (Sigma, St. Louis). The sections were then deparaffinized in xylene and exposed to absolute alcohol for 3 minutes, then 95% alcohol for 3 minutes.

Positive and negative tissue controls were generated from a first-trimester 6 cm crown-rump length bovine fetal testis²¹ and adult rat hearts, respectively. These tissues were dispersed with a 25 mg/100 ml collagenase mixture (Cooper Biomedical, Malvern, Pa.) for 20 minutes at room temperature. After digestion the cells were washed twice with phosphate-buffered saline solution and 5 mmol/L magnesium chloride (Gibco-BRL, Gaithersberg, Md.). Dispersed cells were spun into monolayers and affixed to silane-treated slides with the Cytospin 2 apparatus (Shandon, Pittsburgh). They were then fixed in 4% paraformaldehyde (Sigma) for 10 minutes at room temperature. This was followed by two 15-minute washes in phosphate-buffered saline solution.

To destroy endogenous alkaline phosphatase activity and make the mRNA accessible, affixed tissue sections and control cell monolayers were exposed to 1 µg/ml proteinase K (Boehringer Mannheim Biochemicals, In-

dianapolis) in phosphate-buffered saline solution for 5 minutes at room temperature, washed with 0.2% glycine (Biorad, Richmond, Calif.) in phosphate-buffered saline solution for 10 minutes, and dried completely on a slide warmer at 37° C.

Experimental runs included controls with positive and negative tissue controls, sense oligoprobe, and treatments minus the detection antibody.

Probes. Sense and antisense oligoprobes complementary and unique to the first 27 bases of exon 5 of the human müllerian inhibiting substance gene^{8, 10, 11} were synthesized on an Applied Biosystems, Inc. (Foster City, Calif.) model 380B DNA synthesizer in the laboratory of Terrance Stoming, PhD. These sequences were chosen because they contain an abundance of guanine and cytosine residues,10,11 they have been used successfully for Northern blot analysis by other investigators,8 and are unique to the published human müllerian inhibiting substance gene sequence10,11 according to the Genebank and EMBL databases. A nonradiolabeled detection system was used. With this system a standard reaction with 1 µg of deoxyribonucleic acid (DNA) as template will generate a minimum of 260 ng of labeled probe (Boehringer Mannheim). For this series of experiments end labeling with digoxigenin-11-2'-deoxyuridine 5'-triphosphate (Boehringer Mannheim) was accomplished via terminal transferase (Boehringer Mannheim) at 37° C for 11/2 hours.22 Probes obtained with this procedure can be used to detect about 10 pg of DNA in a dot blot.

Hybridization. Ten microliters of hybridization mixture was added to each slide. The hybridization mixture consisted of 50 µl of deionized formamide, pH 6.8 (Biorad), 20 µl of 50% dextran sulfate (Pharmacia LKB Biotechnology, Piscataway, N.J.), and 10 μl of 20× sodium chloride citrate (SCC) (Sigma), plus 20 mm³ of probe carrier mix. The probe carrier mix consisted of 4 μl of desired probe (500 ng in 20 μl), 8 μl of 0.5 mg/ml salmon sperm DNA (Boehringer Mannheim), and 8 µl of 0.25 mg/ml yeast transfer RNA (Boehringer Mannheim). Silicon-treated coverslips (Sigma) were applied to the specimens, which were placed into hybridization bags (Bethesda Research Laboratory, Bethesda, Md.) and heat sealed. The tissue with added probe was heated for 10 minutes in an 85° C water bath, then allowed to hybridize in a humidified 40° C oven for 18 hours.28 Coverslips were removed by soaking the specimen in $2 \times SCC$ ($1 \times SCC = 0.15 \text{ mol/L}$ sodium chloride and 0.015 mol/L sodium citrate). Unhybridized probe was removed by a series of graded salt washes at room temperature: twice with 2× SCC/Brij for 3 minutes, twice with 0.2× SCC/Brij for 3 minutes, twice with 0.16× SCC/Brij for 3 minutes, and with a final wash of 2 × SCC for 1 minute. Brij (Calbiochem, La Jolla, Calif.) is 2.5 ml/L of 30% Brij 35).23 Constant conditions were maintained throughout all experi-





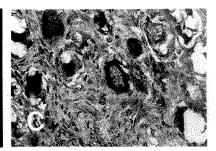


Fig. 2. Same section as in Fig. 1 at higher magnification. Highest staining intensity was noted in primordial oocytes. A, Müllerian inhibiting substance antisense probe. (Original magnification ×10.) B, Matched müllerian inhibiting substance sense probe. (Original magnification ×10.) C, Section shown in A. (Original magnification $\times 160$.)

mental runs except for changes in probe concentration. Probe concentration was manipulated from 10 to 50 ng/μl in an effort to improve signal detection.

Detection. Alkaline phosphatase-conjugated Fab fragments from digoxigenin-induced immunoglobulin in sheep were used to detect hybridized fragments labeled with digoxigenin. In the presence of nitro blue tetrazolium chloride (Sigma) and 5-bromo-4-chloro-3indolyl phosphate (Sigma) a blue-purple reaction product is generated. The specimens were blocked with 3% bovine serum albumin in Trizma-saline buffer (Sigma), washed for five minutes in Trizma-saline buffer, air dried, and exposed to either a 1:500 dilution of antidigoxigenin antibody (Boehringer Mannheim) or control normal sheep serum (Cambridge Research Laboratories, Cambridge, Mass.). Antibody dilutions were carried out for 2 hours at room temperature in humidity chambers. One microliter of antibody was added to a mixture of 20 mg bovine serum albumin, 500 µl Trizma-saline (pH 7.5), and Tween 20 (Sigma), total volume 1.25%.23 The specimens were then washed three times for 3 minutes each in Trizma-saline, pH 7.5, containing 0.1% Triton X-100 (Sigma). These washes were followed by two 5-minute washes in Trizma-saline, pH 9.5 (50 mmol/L magnesium chloride).

Color development was completed under dark conditions by exposure to McGadey's reagent mixture for 2 hours. This mixture consisted of 0.2 ml (50 mg nitro blue tetrazolium chloride per milliliter 50% deionized formamide), 0.1 ml (50 mg 5-bromo-4-chloro-3-indolyl phosphate per milliliter 50% deionized formamide), and 30 ml Trizma-saline, pH 9.5. The sections were then counterstained with nuclear fast red, dehydrated, and mounted with Cytoseal 60 (Stephens Scientific, Denville, N.J.).

Results

Unexpectedly, the most intense signal generated in the ovary was noted within the nuclei of several resting

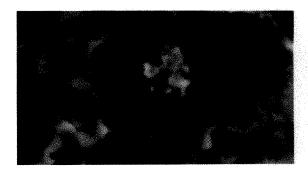


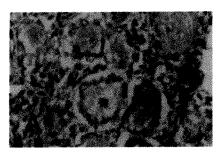
Fig. 3. Different section from same ovary showing two adjacent primordial follicles exposed to müllerian inhibiting substance antisense probe. Pregranulosa cells appear red with staining. Follicle on right demonstrates selective staining confined to cytoplasm. Adjacent follicle demonstrates both nuclear and cytoplasmic staining. (Nuclear fast red. Original magnification ×400.)

primordial oocytes (Figs. 1 through 3). Positive staining was also noted within the cytoplasm of oocytes exposed to the antisense probe. The highest level of cytoplasmic staining was seen in oocytes from primordial follicles. Oocytes within antral follicles from the same tissue sections appeared to have a much lower level of reaction product (Figs. 1 through 4). Semiquantitation for comparison was not attempted, however, because of the small number of antral follicles in the specimen studied.

A lower level of staining was detected within granulosa cells and the ovarian stroma (Figs. 1 through 4). All ovarian cells had some detectable reaction product except for endothelial cells lining blood vessels. This reaction product did not appear to be artifact because matched sense probe sections were negative. An identical staining pattern was demonstrated with the fresh ovarian specimen. The antisense probe stained the bovine fetal testis-positive tissue control more intensely than any of the ovarian sections (Fig. 5). No staining was noted in the adult rat heart-negative tissue control (not shown). As expected, none of the tissue sections or tissue controls stained positively with the sense probe or with the absence of detection antibody.

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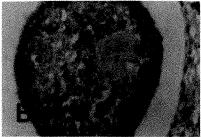
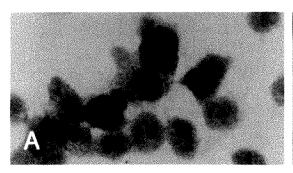




Fig. 4. Section from 40-week human fetal ovary. Probe concentrations were 10 ng per section. A, Müllerian inhibiting substance antisense probe hybridized to stroma and oocyte cytoplasm as demonstrated by blue reaction product. (Original magnification \times 160.) B, Antral follicle from same ovarian section. Oocyte is surrounded by cumulus cells. Both cell types demonstrate low-level expression. (Original magnification \times 160.) C, Matched sense probe. (Original magnification \times 10.)



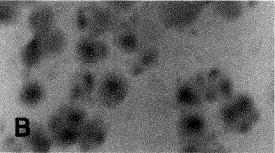


Fig. 5. Positive tissue controls. Probe concentrations were 10 ng per section. Negative tissue controls not shown. A, Bovine fetal testis Cytospin preparation stained positively with müllerian inhibiting substance antisense probe hybridization. (Original magnification $\times 400$.) B, Bovine fetal testis Cytospin preparation did not stain with müllerian inhibiting substance sense probe hybridization. (Original magnification $\times 400$.)

Comment

Utilizing Northern blot analysis, Voutilainen and Miller⁸ detected small, unchanging amounts of human müllerian inhibiting substance mRNA in the extracted RNA from ovaries of 14- to 21.5-week human fetuses. They⁸ further demonstrated that cells from follicular aspirates obtained during oocyte retrieval for in vitro fertilization transcribe müllerian inhibiting substance mRNA in a hormonally responsive fashion. Our work confirms the fact that a small amount of müllerian inhibiting substance transcript is generated by cells in the female gonad throughout human life. It appears, however, that several ovarian cell types participate in this transcription.

One site of localization was the granulosa cells. This was not unexpected because several investigators^{7, 24, 25} have demonstrated immunoreactive müllerian inhibiting substance protein in the granulosa cells of growing follicles. However, müllerian inhibiting substance transcript also was detected in oocytes and ovarian stromal cells. It is doubtful that this localization is the result of nonspecific binding because the appropriate hybridization signals were generated in control tissues and

short oligoprobes specific to the published sequence were used to reduce hybridization to undesired transcripts sharing close sequence homology to müllerian inhibiting substance mRNA.²⁶ Because of institutional restrictions on human fetal research, we used archival tissue. Special care was used to select tissue blocks that were well preserved. Although it is possible that ribonuclease digestion occurred before tissue preservation, the use of short oligoprobes to detect transcript makes it possible to identify specific messages in spite of less than ideal conditions.²⁶ We have avoided attempts to quantify the amount of transcript detected because of possible variations in tissue handling and nucleic acid retention in archival specimens.

Both granulosa and ovarian stromal cells share embryologic origins from mesonephric-derived rete cells destined to participate in ovarian steroidogenic function.²⁷ Granulosa cells demonstrate müllerian inhibiting substance gene expression subject to cell-specific regulation with the production of müllerian inhibiting substance protein.⁷ ⁸ Oocytes, on the other hand, are descendants of primitive germ cells and serve a totally different function.²⁸ These cells must establish genetic

variation, generate many of the substances necessary for oocyte fertilization, and store materials for future support and regulation of the preimplantation conceptus.28 Active müllerian inhibiting substance transcription within the oocyte suggests some physiologic role. Whether this role involves autocrine regulation of oocyte competence is speculative. If müllerian inhibiting substance protein is involved in the maintenance of meiotic arrest as proposed by Takahashi et al,14 one might expect to detect the most significant levels of ovarian müllerian inhibiting substance protein production during the time when most oocytes are present in the dictyotene stage. This would be during the latter part of human fetal development. 19, 28 To date immunoreactive müllerian inhibiting substance protein has not been detected in any mammalian oocytes. This could be due to the fact that the amounts of translated protein may be too small to detect with generated müllerian inhibiting substance antibodies or to epitope differences between the fetal and adult product. However, it is quite possible that oocyte müllerian inhibiting substance transcript is not translated until after fertilization events take place. The oocytes of many species have been shown to store deadenylated RNA for subsequent use after fertilization.29

It is interesting that müllerian inhibiting substance belongs to the transforming growth factor-\beta family of proteins, 10, 30 which includes the Vg1 gene product, an oocyte protein associated with mesoderm induction during early Xenopus development.30 Could it be that oocyte müllerian inhibiting substance transcript, like several other transforming growth factor-\beta family members, plays some role in the diversification of cell fate during embryonic cleavage? Further investigations are currently under way to elucidate this most interesting possibility.

We acknowledge Terrance A. Stoming, PhD, of the Department of Biochemistry and Molecular Biology at the Medical College of Georgia for synthesizing the oligoprobes used in this study; Susan Malone and William Wamsley for help with tissue block sectioning; and Paul G. McDonough, MD, for critical review of the manuscript, helpful comments, and sponsoring the abstract.

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The presence of the testicular determining sequence, SRY, in 46,XY females with gonadal dysgenesis (Swyer syndrome)

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Subjects with 46,XY gonadal dysgenesis (Swyer syndrome) have a distinctive phenotype. They are normal or tall in stature, lack somatic anomalies, and possess bilateral rudimentary gonads. Critical Yp deletions have been described in some cases, but in the majority no defects at the molecular level have been reported. To verify the presence or absence of SRY, the putative testicular-determining factor gene, specific primers were designed to amplify the conserved region of the SRY gene. Deoxyribonucleic acid from control males (n=10) and sex-reversed females with the Swyer syndrome phenotype (n=5) generated the anticipated 310 bp band. This Y-specific band was absent in the deoxyribonucleic acid from control females (n=9). To search for possible point mutations, the amplified products of all study subjects and one control male were sequenced in both orientations. The base pair sequences were all identical and similar to the previously published report. (AM J OBSTET GYNECOL 1991;165:1887-90.)

Key words: Testicular determining factor, sex-reversed females, pure gonadal dysgenesis, Swyer syndrome

In man, differentiation of the testis from the embryonic biopotential gonad (genital ridge) is primarily initiated by the expression of a Y-associated gene(s) called the testicular-determining factor.1-7 This putative factor probably activates a succession of autosomal genes that switch the inherently female pattern of development to that of the male, beginning with testis formation followed by production of müllerian-inhibiting factor and testosterone. The assignment of testicular-determining factor to the Y-chromosome short arm has been possible by studying sex-reversed individuals—XY females with Yp deletions and XX males with Y/X translocation chromosomes. 1. 3-6 By deletion analysis of an XX male carrying the distal part of the Y short arm and a female with a Y-autosomal translocation missing the same region, Page et al.4 isolated a 140 kb fragment of the Y chromosome that encodes a "zinc finger" protein. The gene, designated ZFY, which is highly conserved among mammals, became the prime candidate for testicular-determining factor. Later, however, three XX males and one XX intersex individual were discovered who are missing ZFY and instead carry a fragment of Y deoxyribonucleic acid (DNA) distal to the ZFY locus.⁶ A 35 kb segment was cloned from the region that seemed to contain the essential element(s) required to initiate male sex determination. In this 35 kb region, so far only one gene, designated SRY, has been located. This gene is Y specific, highly conserved among mammals, and transcribed only in testis.7 The predicted amino acid sequence of SRY shares homology with DNA-binding domains of transcription factors such as chromatin-associated, nonhistone proteins HMG 1 and HMG 2. Gubbay et al.8 cloned the SRY-related gene Sry from the mouse Y chromosome and showed that it is exclusively expressed in the mouse embryonic gonad. Most recently a 14 kb mouse Y-chromosomal DNA that carries the Sry gene region was introduced into mouse embryos to obtain transgenic animals that could express and vertically transmit the integrated Sry gene. Three of 11 XX transgenic animals showed sex-reversed phenotypes, which argues that the 14 kb fragment includes the essential information required for male sex determination in the mouse. However, when a 25 kb human Y-DNA fragment including the SRY gene was introduced, the transgenic animals showed no sex reversal.9

Although *Sry* seems to fulfill the criteria for the testisdetermining gene, further analysis of the human SRY gene and studies of phenotype-genotype correlations in subjects with abnormal sex differentiation will help to better clarify the mechanism of action of regulatory and structural genes involved in sex differentiation. In this regard testing the integrity of the SRY gene in 46,XY females is of considerable interest. In this study the DNA of a group of 46,XY females, carefully selected for the Swyer syndrome phenotype and devoid of all somatic anomalies, was analyzed for the presence of SRY and its integrity. Such sex-reversed females

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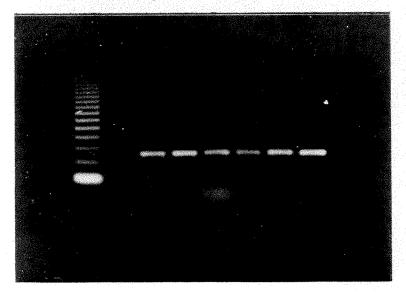


Fig. 1. Polymerase chain reaction amplification of SRY sequence from five patients with Swyer syndrome as compared with normal male (M) and female (F) samples. The 310 bp polymerase chain reaction products are stained with ethidium bromide in 1.2% agarose gel. Band is absent in control female sample. SRY primers 5'-GTGTGAAACGGGAGAAAA CAGTAAA-3' and 5'-ATCTGCGGGAAGCAAACTG-3' were synthesized with a Pharmacia-LKB Gene Assembler Plus (Piscataway, N.J.). Reaction was carried out for 30 cycles at annealing temperature of 55° C, and 8% of end products were used for electrophoresis.

could have simple mutations in the SRY gene; alternatively, they could have mutations that block gene actions further upstream or downstream in the cascade responsible for male differentiation.

Material and methods

Patients. Written informed consent was obtained from each patient participating in the study, as approved by the Human Assurance Committee at the Medical College of Georgia. The 46,XY sex-reversed females (n = 5) selected for this study were all sporadic cases, devoid of somatic, cardiac, or renal anomalies, and ranged in adult height frrom 167 to 190 cm (mean 173.8 cm). Serum gonadotropin levels were markedly elevated in all five subjects. Peripheral blood karyotyping with 50 cells counted revealed a uniform 46,XY karyotype with a structurally normal Y chromosome on G, Q, and C banding. Bilateral rudimentary streak gonads were present at laparotomy in all cases, except for three where they were unilaterally replaced by tumor.

DNA analysis by polymerase chain reaction amplification. The DNA of the study subjects was analyzed by the polymerase chain reaction for the presence or absence of SRY. Nucleotide positions of primers relative to the sequence published by Sinclair et al.7 were 543 to 569 for 5', and 833 to 854 for 3', flanking a 312 bp segment, including the coding domain of the SRY gene. DNA samples from various sources such as blood lymphocytes, skin fibroblasts, or Epstein-Barr virustransfected lymphoblastoid cells were examined from each individual, depending on availability. The polymerase chain reaction was carried out in a total volume of 100 µl with 1 µg of DNA in 10 mmol/L Tris, pH 8.3, 1.2 mmol/L magnesium chloride, 50 mmol/L potassium chloride, 0.01% gelatin, 250 µmol/L each dNTP, and 3.3 units of Taq polymerase (Thermus aquaticus; Perkin Elmer/Cetus, Norwalk, Conn.) in the presence of 0.5 µmol/L primers. In the initial cycle denaturation was at 96° C, annealing at 55° C, and extension at 72° C, each for 5 minutes. This was followed by 30 cycles of 3, 2, and 5 minutes for each denaturation, annealing, and extension step, respectively, with a final extension step of 10 minutes in the last cycle.

Asymmetric polymerase chain reaction and DNA sequencing. For sequencing, the SRY fragment was amplified from each DNA sample as above. One microliter of each of the end products was transferred to a freshly prepared polymerase chain reaction containing only one of the primers, and the amplification reaction was 5'-gtgtgaarc ggagaarca gtaaaggca cgtccagat agagtgaagc gacccatgaa CGCATTCATC GTGTGGTCTC GCGATCAGAG GCGCAAGATG GCTCTAGAGA ATCCCAGAAT GCGAAACTCA GAGATCAGCA AGCAGCTGGG ATACCAGTGG ARAATGCTTA CTGAAGCCGA AAAATGGCCA TTCTTCCAGG AGGCACAGAA ATTACAGGCC ATGCACAGAG AGAAATACCC GAATTATAAG TATCGACCTC GTCGGAAGGC GAAGATGCTG CCGAAGAATT GCAGTTTGCT TCCCGCAGat-3'

Fig. 2. Nucleotide sequence of 310 SRY coding domain amplified from five sex-reversed females and one normal male. Asymmetric polymerase chain reaction was performed for 50 cycles with 1 mm³ of polymerization products described in Fig. 1 as template, with the addition of only either forward or reverse primers. Purified asymmetric polymerase chain reaction products were then used for sequencing by standard dideoxy termination method of Sanger with the Sequenase II kit. *Arrows* show primers and direction of amplification. *Bold uppercase letters* show sequenced nucleotides, whereas *lowercase letters* indicate nucleotides that were present in primers but were not sequenced.

carried out for 50 cycles to generate single-stranded DNA of both orientations. DNA sequencing was performed with the Sequenase kit (USB Co., Cleveland).

Results

All of the subjects and 10 male control DNA samples were positive whereas nine female control samples were negative for the 312 bp band that represents the coding sequence of SRY (Fig. 1). Samples from normal females occasionally formed a very weak band of lower molecular weight, but none of them revealed the specific band found in males and in the 46,XY sex-reversed females studied. Since SRY codes for a conserved 80 amino acid motif with great homology with a family of transcription factors encoded by autosomes, we sequenced the amplified fragments from all patients and one normal male to confirm that the amplified regions actually represent the Y-chromosome sequence. The results precisely matched the sequence published by Sinclair et al.7 (Fig. 2). These data also rule out the presence of a point mutation in the coding domain of the SRY gene as a possible cause of sex reversal in these XY females.

Comment

Although SRY appears to fulfill the criteria for the testis-determining gene, it has not been excluded that some other gene may exist in the 35 kb Y fragment found in the XX males studied by Sinclair et al. and Berta et al. Furthermore, although testes developed in these ZFY-negative XX males, male genitalia were not fully developed. In the mouse the sex-determining region of the Y is now clearly defined within a 14 kb fragment capable of rendering the XX transgenic animals male; however, it is not clear why only three of 11 transgenic animals showed sex-reversed phenotypes. Furthermore, none of the transgenic animals carrying the 25 kb fragment, including the human SRY, showed sex reversal.

If SRY is testicular-determining factor, 46-XY sexreversed females with intact Y chromosomes may have missing or mutated SRY sequences. Berta et al.10 examined the SRY DNA sequence in 11 sex-reversed females and found only single-base substitutions in two of them $(G \rightarrow A \text{ in one and } G \rightarrow C \text{ in the other})$. These base substitutions, however, are known to cause conservative amino acid changes. Notably, the normal father of one of these subjects was carrying the same single base substitution in his SRY.10 Jager et al.11 analyzed 12 sex-reversed females and found a frameshift mutation in one of them. This individual is tall in stature and has a 46,XY karotype and no evidence of mosaicism. To our knowledge, this is the only case reported with detailed ascertainment of pure gonadal dysgenesis in an individual with a defined mutation in SRY. Subjects with pure 46,XY gonadal dysgenesis (Swyer syndrome), if appropriately selected, should have a solitary anomaly, the failure of gonadal development. The gonads in patients with Swyer syndrome are undifferentiated streaks composed of fibrous tissue and devoid of germ cells. Because of the lack of gonadal steroids that allow delayed epiphyseal closure, the height of Swyer syndrome subjects is normal or increased, as compared with their 46,XX siblings, which distinguishes this syndrome from other forms of gonadal dysgenesis with the somatic signs of Turner syndrome associated with X monosomy or 45,X/46,XY mosaicism. Deletions of Yp have been described in some nonmosaic, 46,XY sex-reversed females. However, the presence of short stature and somatic anomalies in most of these reported cases suggests mosaic 45,X/46,XY gonadal dysgenesis rather than Swyer syndrome.1.3,12,13 According to the X,Y interchange model, the XY females should lack some terminal portion of Yp. Previously we have reported the presence of ZFY in these subjects,14 and the integrity of the new candidate sex-determining locus, SRY, is demonstrated here. Whether the SRY gene in these pure 46,XY subjects is inactive or they have other unidentified deletions remains to be seen. The presence of families in which Swyer syndrome appears to show X-linked inheritance¹⁸ also raises the question as to whether an X-linked gene that functions downstream from SRY is involved in creating the Swyer syndrome phenotype. These subjects, when carefully selected for their distinctive phenotype, provide the best opportunity to study the aberrations of gonadal development in man.

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Evidence for a partial deletion in the androgen receptor gene in a phenotypic male with azoospermia

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Androgen resistance is thought to vary phenotypically from a normal female to an infertile male. Previous evaluation of infertile males has been limited to androgen receptor—binding affinity. The androgen receptor gene has been isolated, cloned, and studied extensively in patients with complete androgen insensitivity syndrome, but no comparative data are available on infertile males. To address this matter, the androgen receptor gene was studied in seven azoospermic males by use of the polymerase chain reaction and Southern blot hybridization. A partial gene deletion was found in one patient. This study provides the first molecular evidence of an abnormality in the androgen receptor gene in a phenotypic male with azoospermia. (AM J OBSTET GYNECOL 1991;165:1891-4.)

Key words: Male infertility, azoospermia, androgen receptor, androgen insensitivity syndrome

The range of phenotypes in subjects with androgen resistance may vary from 46,XY sex-reversed females to undermasculinized normal males. Aiman et al.1 published a report in 1979 suggesting that phenotypically normal, infertile males also may be included in the spectrum of androgen insensitivity. They described 3 phenotypically normal men with semen abnormalities (2 azoospermic and 1 oligospermic) as having abnormal androgen receptor-binding affinity. A follow-up study by the same group2 reported on 22 phenotypically normal men with either idiopathic azoospermia or severe oligospermia. Abnormal androgen receptor-binding affinity was seen in 9 of 22 patients (8 azoospermic and 1 oligospermic). In 3 of the 9 patients with low androgen receptor activity the serum testosterone levels were elevated above normal. Their conclusions suggested that ≥40% of men with male infertility that is a secondary effect of azoospermia or severe oligospermia may have some degree of androgen resistance.

Bouchard et al.³ questioned this high prevalence of androgen resistance as a cause of infertility in males with normal phenotypes. Their study included 24 men with severe oligospermia. The testosterone values were all within the normal range. Fibroblasts were cultured and androgen receptor—binding affinities were normal in all patients. However, no patients with azoospermia were included in their report.

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Recent studies of androgen insensitivity syndrome have focused at the molecular level and limited the study population to subjects with complete androgen resistance. In 1988, Lubahn et al.4 reported the isolation and cloning of the gene for the androgen receptor. Shortly thereafter, the same group and others described the use of radiolabeled probes on Southern blots prepared by restriction endonuclease digestion of genomic deoxyribonucleic acid (DNA) to detect partial androgen receptor gene deletions in individuals with complete androgen insensitivity syndrome.5,6 This was followed by publication of the entire sequence of the eight exons present in the androgen receptor gene along with their intron-exon borders. Subsequent nucleotide sequencing of the androgen receptor gene in one patient with complete androgen insensitivity syndrome revealed a single point mutation.7 At least one additional article also describes the presence of a point mutation in a patient with complete androgen insensitivity syndrome.8 These studies have served to increase our understanding of the etiology of complete androgen insensitivity syndrome.

No comparative data at the molecular level are available on phenotypically normal males who might have infertility as a secondary effect of some degree of androgen resistance. The purpose of this study was to search for molecular evidence that might support this hypothesis.

Material and methods

Approval was given by the Human Assurance Committee at the Medical College of Georgia, and informed written consent was obtained from patients (n = 7) and controls (n = 1) to participate in the study. Peripheral

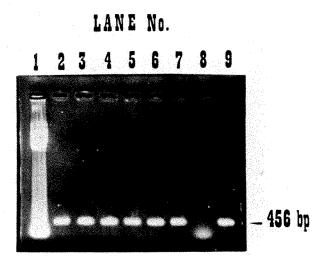


Fig. 1. Polymerase chain amplification of exon 4 of androgen receptor gene in azoospermic males. *Lane 1*, DNA ladder (multiples of 123 bp); *lane 2*, control male; *lanes 3 to 9*, azoospermic patients. *Lane 8* demonstrates absent band corresponding to exon 4.

blood was obtained by venipuncture, and DNA extraction was accomplished by standard techniques. Each patient had presented with infertility as a result of azoospermia. Clinical evaluation included a normal male phenotype, a 46,XY karyotype, including a morphologically normal Y, a normal serum testosterone level (300 to 800 ng/dl), a normal serum luteinizing hormone level (5 to 20 mIU/ml), a vasogram showing patency, and a testicular biopsy specimen revealing either germ cell aplasia or maturation arrest.

Polymerase chain reaction. According to the published sequence for the exons and immediately flanking introns of the androgen receptor gene, corresponding oligonucleotide primers identical to those used by Lubahn et al.⁷ were synthesized for each of the exons on the Gene Assembler Plus (Pharmacia). The primers ranged in size from 20 to 30 nucleotides. Purification of the primers was achieved by filtration through a Sephadex G-25 (Pharmacia) column.

The polymerase chain reaction was performed on each patient and control. Genomic DNA (1 µg) was added to a solution of reaction buffer consisting of 50 mmol/L potassium chloride, 10 mmol/L Tris hydrochloride (pH 8.3), 1.5 mmol/L magnesium hydrochloride, and 0.01% gelatin. Oligonucleotide primers (20 mmol/L), deoxyribonucleoside triphosphates (200 µmol/L each), and *Thermus aquaticus* DNA polymerase (1.5 units) were then added. Amplification was carried out on a DNA thermal cycler with initial denaturation at 95° C for 5 minutes, annealing at 55° C for 5 minutes, and polymerization at 72° C for 5 minutes. Next, 30 cycles of 1 minute at 95° C, 1 minute at 55°

C, and 1 minute at 72° C were performed followed by a final 5 minutes at 72° C. After amplification, the products were evaluated by size by means of gel electrophoresis.

Southern analysis. The probe hAR1, which is specific for the androgen receptor gene, was used for Southern analysis. It is a 718 bp EcoRI/HindIII complementary DNA (cDNA) fragment that contains a portion of the DNA binding domain at its 5'-terminus and extends into the 3'-steroid-binding domain (for map see reference 5). Genomic DNA digestion in normal patients yields two EcoRI fragments of 9.4 and 2.4 kb on Southern blots. The 9.4 kb fragment is thought to contain the information for exon 4.5 The probe was originally derived from clone ARHFLIH-X obtained from a human foreskin fibroblast cDNA library and inserted into a Bluescript SK+ vector.4 The plasmid was amplified in Escherichia coli and purified by cesium chloride gradient. The corresponding insert was released from the vector and labeled by oligonucleotide random priming to a high specific activity of 1×10^8 counts/min/ μ g DNA with the procedure recommended by the supplier (Amersham).

Genomic DNA from both the patients and the control was digested with EcoRI restriction endonuclease and samples of 10 µg each were electrophoresed in a 0.8% agarose gel. DNA was then transferred to Gene Screen Plus (New England Nuclear Research Products, Boston) by the Southern method¹⁰ and baked in a vacuum oven for 2 hours at 80° C. The blot was then hybridized for 48 hours at 65° C with a phosphorus 32–labeled probe and washed in high-stringency conditions at 65° C in 0.1 × sodium chloride—sodium citrate and 1% sodium dodecyl sulfate. Autoradiographs were exposed with intensifying screens at -80° C for 3 days on Kodak XAR-5 film (Eastman Kodak Company, Rochester, N.Y.).

Results

Evaluation of polymerase chain reaction—amplified exons, through the use of gel electrophoresis to determine product size, revealed no gross abnormalities in each of the 7 patients for exons 2 through 8 with only one exception. The product corresponding to exon 4 was absent in 1 patient (Fig. 1).

Southern analysis confirmed an abnormality in this same region of the androgen receptor gene as evidenced by an absent 9.4 kb band in the affected patient as compared with a control male (Fig. 2).

Comment

Aiman et al. first presented their theory of androgen resistance as a cause of male infertility in patients with normal phenotypes in 1979 and later studies have sup-

ported their hypothesis. Smallridge et al.¹¹ described oligospermic monozygotic twin brothers with normal male phenotypes who had decreased androgen receptor activity. This observation would imply a genetic basis for the oligospermia.

These reports suggest that androgen resistance is a cause for infertility; however, our study is the first to provide direct evidence for this hypothesis at the molecular level. Through the use of the polymerase chain reaction, an absent band corresponding to exon 4 of the androgen receptor gene in one azoospermic male was detected. The presence of this deletion is confirmed by the absence of the 9.4 kb band on the Southern analysis.

Exon 4 is thought to encode for the part of the androgen receptor protein that facilitates its movement into the nucleus of the target cell after binding to the steroid hormone. This hypothesis is based on in vitro studies in which induced mutations within exon 4 have been shown to interfere with cytoplasmic-to-nuclear migration of the androgen receptor-steroid complex but not the actual binding of the steroid hormone to the receptor. It is conceivable that the nuclear transfer of steroid-receptor complex in our affected subject is less efficient, resulting in lower androgen-dependent transcriptional activity of some target genes. This activity may be adequate for the male phenotypic expression but inadequate for proper germ cell function resulting in azoospermia.

We are aware of only two studies of complete androgen insensitivity syndrome where mutations involving exon 4 have been reported. The first describes a point mutation in the intron between exons 4 and 5 involving the splice donor site of intron 4. This abnormality is thought to result in a truncated messenger ribonucleic acid at the splice site and does not allow translation of exons 5 to 8. The second article describes three complete androgen insensitivity syndrome siblings, each carrying the same single point mutation within exon 4. This latter point mutation changes a tryptophan to a translation stop codon, again creating a truncated androgen receptor lacking most of its steroid-binding domain.

Complete exon deletions may result in frameshift errors that interfere with the translation of downstream exons, resulting in nonfunctional proteins. The dystrophin gene in most patients with Duchenne muscular dystrophy contains such a deletion. A resultant truncated protein is responsible for the severity of this disease. Conversely, patients with Becker muscular dystrophy frequently have a dystrophin gene exon deletion that does not result in a frame shift error. The downstream exons are still translated and the resultant protein, although altered, still has some normal func-

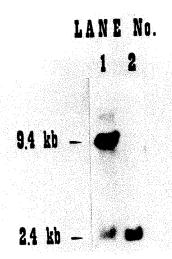


Fig. 2. Southern blot of genomic DNA digested with *Eco*RI restriction endonuclease. Hybridization with probe hAR1 reveals absent band in affected patient (*lane 2*) versus control in *lane 1*.

tion. Hence Becker muscular dystrophy is a much less severe disease. 15, 16

The published sequence of the entire androgen receptor gene? indicates that complete deletions of either exon 3 or 4 will not result in a frameshift error of the exons downstream. However, deletions of any of the other exons will result in a frameshift error that would be manifested at the time of translation. These observations further support our findings of a deleted exon 4 in an azoospermic male. Exons 5 to 8, which make up the bulk of the steroid-binding domain, 7. 12 would thus be intact and available for proper translation allowing for the male phenotype. However, as discussed previously, the amount of receptor-steroid complex reaching the target cell nucleus may be decreased and inadequate for spermatogenesis. Future study of this patient in question may be further revealing.

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LETTERS TO THE EDITORS

Giacomello's observation and nuchal cords

To the Editors: In Giacomello's letter (Giacomello F. Ultrasound determination of nuchal cord in breech presentation. Am J OBSTET GYNECOL 1988;159:531-2) a description of an umbilical cord pattern relative to the fetal neck was illustrated. Giacomello warned that before initiating version on a breech fetus physicians should be aware of the possibility of nuchal cord tightening. He did not mention the quantitation of this pattern. Patterns A and B were studied in 288 deliveries. Careful attention was paid to the cross-over versus cross-under forms. Thirty-nine nuchal cords were delineated, and all crossed over the umbilical end (pattern A). Although a cross-under form (pattern B) was not observed, it is the only pattern that can create a true knot. That there were 11 true knots in this series suggests that pattern A is more common than pattern B and that fetal rotation on its long axis (360 degrees) is the predominant mechanism of nuchal cords; however, it does not suggest that pattern B does not exist in the form of a nuchal cord even though pattern B was not observed in this series.

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Reply

To the Editors: We are grateful for the opportunity to reply to the interesting comments regarding our letter. Collins reports his data on 288 deliveries concerning the incidence of umbilical cord knots and of the nuchal cord patterns shown in Fig. 1 accompanying our letter. Although pattern B was not recorded, pattern A and true knots were observed in 39 (13.5%) and 11 (3.8%) cases, respectively. The incidence of cord knots has been reported to be from 0.3% to 2.1% and that of encirclements from 15.8% to 30%, but a wider range is possible, especially in small series. Recently, two consecutive cases of midtrimester abortion occurring at 16 and 18 weeks' gestation in association with cord encirclement of the fetal neck were recorded in our institution; in the second case pattern B was observed. In our letter the pattern of cord encirclement was not recorded because our observations did not focus on this issue. We agree with Collins, whose data suggested that "fetal rotation on its long axis (360 degrees) is the predominant mechanism of nuchal cords." On the other hand, pattern B can be considered to be an incomplete knot; its rarity at birth could support the common belief that cord knotting occurs within the first trimester because of the peculiar proportion of the small fetus (big head compared with the body) moving in a relatively large amount of amniotic fluid. In addition, pattern B is more dangerous for the fetus than a complete knot because the cord can tighten very easily

and lead to fetal miscarriage in the second trimester, as was observed in our second case.

We have not suggested that nuchal cord tightening during external cephalic version could be related to pattern B. Other factors such as a short cord or multiple encirclements could be involved. The interesting point was that in breech delivery pattern B, even if uncommon, tightens and pattern A may easily unroll and not be recorded at birth. However, in cephalic presentation intrauterine cord encirclements of the fetal neck are usually recorded at birth, at least with normal fetal rotation.

The increased safety and success rate of external cephalic version can be attributed, at least in part, to correct patient selection on the basis of clinical and ultrasonographic data and fetal heart rate monitoring. The ultrasonographic evidence of multiple nuchal cords and neck hyperextension could advise against too vigorous attempts at cephalic version to avoid complications.

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Ethics in medical studies with human subjects

To the Editors: We read with interest the recent article by Goldstein et al. (Goldstein I, Zimmer EZ, Merzbach D, Peretz BA, Paldi E. Intraamniotic infection in the very early phase of the second trimester. Am J Obstet Gynecol 1990;163:1261-3) in which they concluded that intraamniotic infection may exist early in a pregnancy with intact membranes. The findings of this and similar studies are fascinating and may shed light on the causes of otherwise unexplained spontaneous abortions or preterm contractions.

However, the results as described with evidence of a 50% perinatal complication rate (preterm delivery and septic abortion) do not in our minds represent a "relatively good outcome" and lead to these questions:

- 1. Was this study authorized or reviewed by an institutional ethics committee?
- 2. Were the patients aware that amniotic fluid cultures were obtained?
- 3. Did the patients give their consent before participating in the study?
- 4. In the cases with positive cultures, were the patients or attending physicians informed of the findings and consulted as to management; that is, was it considered that infection caused by pathogenic organisms such as *Klebsiella* and *Escherichia coli* or group B β -hemolytic streptococcus might be treated with antibiotic regimens?

In this day of active patient involvement in medical care and the current medicolegal atmosphere, these ethical issues are essential; if they were addressed by the authors, it was not clearly stated in the text.

David M. Sherer, MD, and Jacques S. Abramowicz, MD Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Strong Memorial Hospital, The University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Box 668, Rochester, NY 14642

Reply

To the Editors: We thank Sherer and Abramowicz for their interest in our study. The study was approved, patients' consent was obtained before amniocentesis, and patients were aware that amniotic fluid cultures were obtained. We are unaware of similar studies at such an early gestational age; to the best of our knowledge there are no data available as to how these cases should be treated. Patients were monitored in our outpatient clinic but were not treated with antibiotics. On the basis of the data obtained from our initial study, we are designing a new study in which patients with positive cultures will be offered treatment with antibiotics. The mode and duration of treatment and the possible need for a second amniocentesis after completion of treatment have still to be evaluated.

Israel Goldstein, MD, and Etan Zimmer, MD Department of Obstetrics and Gynecology "B," Rambam Medical Center, Haifa, Israel

Factors affecting embryo implantation after human in vitro fertilization

To the Editors: Paulson et al. (Paulson RJ, Sauer MV, Lobo RA. Factors affecting embryo implantation after human in vitro fertilization: a hypothesis. Am J Obstet Gynecol 1990;163:2020-3) present a model for attempting to define some very difficult questions regarding oocyte-embryo quality and endometrial receptivity in in vitro fertilization (IVF). I take issue with their use of the model and some of the conclusions derived from it.

In comparing their oocyte donation and regular IVF results, they made a relatively small number of observations to obtain the percentages of implantation for each group. If the 95% confidence limits were given for each of these, the reader would realize the very wide ranges within which the true values could lie based on the *n* of the samples used. These inaccuracies could profoundly influence the difference between the two rates, which was the basis for the estimation that receptivity is reduced during stimulated cycles by 67%. For example, if the two rates were 25% and 15%, receptivity would be reduced by only 40%.

The authors used the difference between these two implantation rates to conclude that endometrial receptivity is dramatically reduced in the stimulated cycle. Their conclusion is based on the critical assumption that egg quality was equal in the two groups. However, the egg donors were normal women. If they were younger

(mean ages were not given) or inherently more fertile than the women needing IVF, their oocyte quality could have been unusually high relative to that of the women needing IVF. Unless all of the IVF women had tubal occlusion from the beginning of their attempts at fertility, they could represent a subgroup of women who because of ovarian or uterine factors are less fertile than the total population of women with tubal disease who attempt pregnancy. The markedly reduced implantation of frozen donor oocytes compared with frozen oocytes from IVF patients also clearly indicates that either oocyte or uterine factors are present in the IVF population.¹

The reduction of the implantation rate in the IVF women could be caused by uterine factors other than the excess stimulation of the endometrium. These women differed from the egg recipients by having had pelvic infection. For example, implantation has been shown to be lower in women with positive titers for chlamydia.² The likelihood of the reduction being entirely caused by stimulation is certainly lessened by the authors' observation that implantation was increased with a greater level of stimulation.

The model proposed is a reasonable one to use in trying to define these difficult questions. However, the magnitude and cause of the reduced implantation in women having IVF remain to be defined.

David R. Meldrum, MD

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Reply

To the Editors: In our December 1990 article we presented a formula that attempts to combine available embryo implantation information in a single expression: Embryo implantation rate (EI) = Embryo quality (EQ) × Endometrial receptivity (ER) × Transfer efficiency (TE). This formula allows comparison of any of the three variables (EQ, ER, or TE) among various assisted reproductive techniques. For example, TE may be different if embryos are replaced via fallopian tubes rather than transcervically, ER may be different with different endometrial stimulation regimens, etc. To formulate specific comparisons among different reproductive techniques, the standard algebraic problem of how to obtain values for each variable in equations with multiple independent variables must be solved. The solution is to have as many equations as there are independent variables. This works perfectly in the perfect world of mathematics but only imperfectly in the world of clinical medicine, where no quantitative comparisons are possible unless basic assumptions are made.

For the purpose of the Comment in our article, we assumed that EQ was equal in the donor and standard IVF groups; this is the assumption with which Dr. Meldrum takes issue. His point is well taken; although the groups were as similar as possible, they are technically not equivalent for the reasons he cites. The cohort of patients selected for the standard IVF group performs the best in our hands of all groups of IVF patients, but it may be possible that normal fertile women (who act as oocyte donors but otherwise have no reason to undergo IVF) would perform even better. Thus EQ might be higher in the donor group, and the observed decrease in implantation rates might not be entirely caused by decreased ER in the standard IVF group. We tend to believe that this is not the case and have elaborated on the matching of the two groups more extensively in the original report of those data. A better study design was actually reported by Levran et al.2 who divided embryos produced from a single cohort of oocytes among the donors themselves and two or more recipients. In their series, 12 oocyte aspirations led to 12 donor and 27 recipient embryo transfer procedures. Ten pregnancies resulted, all in the recipients and none in the donors, supporting the contention that (1) the quality of embryos derived in vitro from stimulated cycles is very high, with nearly all oocyte cohorts resulting in pregnancies, and (2) the endometria of patients who undergo controlled ovarian hyperstimulation are less receptive than those of patients who cycle naturally or have exogenously generated cycles.

To further delineate the magnitude of the effect of controlled ovarian hyperstimulation on ER, Dr. Meldrum points out the relatively small numbers of patients in our series. Indeed, these are the 95% confidence intervals, as determined by binomial expansion of EI for the two groups: Donor IVF (n = 75), EI = 35% (95% confidence interval = 24% to 46%); Standard IVF (n = 225), EI = 11% (95% confidence interval = 7% to 15%).

Thus, if we take the low extreme for the donors and the high extreme for the standard group (24% vs 15%), the magnitude of the reduction in ER is smaller (40%) than the 67% calculated on the basis of the observed EI rates. However, by the same argument, if the opposite extremes are used (46% vs 7%), a much higher ER effect (85%) can be calculated. We agree with Dr. Meldrum that the exact magnitude of the reduction in ER cannot be obtained from a single report, especially if this effect is different for different populations, but we believe that the value of 67% is a close approximation.

Finally, Dr. Meldrum quotes our observation³ that very high estradiol levels by themselves are not detrimental to the clinical outcome of IVF. This does not disprove the theory that controlled ovarian hyperstimulation is inhibitory to ER, however. There are many

other factors associated with high estradiol levels, including favorable patient selection (more fertile patients have higher estradiol responses) and greater ability to select embryos. There are other ovarian secretory products associated with controlled ovarian hyperstimulation that may not correlate with estradiol levels; thus inhibition of ER may not correlate with estradiol levels. Our article³ discusses these issues and states only that it is unlikely that the ER effect is mediated by estradiol alone.

We appreciate the opportunity to expound on our model and hope that our formulation will help others explain some of the differences among EI rates that they observe in their own programs. In this way we hope that greater insight may be gained into the process of embryo implantation and energy may be focused on appropriate parts of the puzzle. As of this point we feel that among younger IVF patients ER is the rate-limiting step of success and that if ER can be improved IVF pregnancy rates can be subtantially increased to approximate those of oocyte donation.

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The origin of brain lesions in survivors of twin gestations complicated by fetal death

To the Editors: In their excellent study Bejar et al. (Bejar R, Vigliocco G, Gramajo H, et al. Antenatal origin of neurologic damage in newborn infants. II. Multiple gestations. Am J Obstet Gynecol 1990;162:1230-6) have found a high incidence (30%) of antenatal necrosis of the cerebral white matter in the survivors of monochorionic multiple gestations complicated by fetal death. On the basis of their findings and previous studies, the authors attribute those lesions to thromboemboli from the dead fetus reaching the surviving twin through the placental vascular anastomosis.

Although such a mechanism cannot be ruled out, a cause-and-effect association between the two has never been proved. Other possible explanations as to the high incidence of brain lesions in the survivors include (1) hypoxia and hypotension at the time of the insult that produced the single fetal death and (2) prematurity as a consequence of spontaneous or elective preterm delivery. We' recently reported the outcome of four mon-

ochorionic triplet gestations complicated by fetal death. No evidence for thromboembolic lesions was found in the nine survivors, and their outcome did not differ from that of a control group. In fact, the data presented by Bejar et al. hardly support the thromboembolic theory: Of the 12 monochorionic infants with antenatal necrosis of the cerebral white matter, in eight the lesions were present in both infants, in two only one infant was affected, and in two there was a history of a cotwin dying in utero. On the contrary, their data show that monochorionic placentation per se and not a dead fetus may be responsible for the high incidence of whitematter brain lesions (10/12 cases); the data certainly do not support any cause-and-effect relationship between fetal death and brain lesions in the survivors.

The clinical implication of this discussion is that fetal death in multiple gestation should not be considered an indication for delivery of the surviving fetus on the basis of the assumption that prompt delivery may prevent the development of vascular disruption, which could lead to disease in various organ systems. However, because the factor responsible for the death of one fetus may further jeopardize the survivor, close assessment of fetal well-being is mandatory.

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Response declined

First report: Prenatal diagnosis of a true knot

To the Editors: A true knot of the umbilical cord occurs in approximately 1% of deliveries and leads to a peri-

natal loss of approximately 6%. To date, no published case of a prenatally diagnosed true knot could be located. Fig. 1 is a photograph of a true knot diagnosed at 32 weeks with a model 3000 General Electric ultrasonography unit. This 32-year-old woman, gravida 5, para 2, aborta 2, was evaluated with biweekly nonstress tests. By 36 weeks the fetal heart rate baseline rose from 160 to 180 beats/min. Repeat ultrasonography demonstrated decreased amniotic fluid. A follow-up oxytocin stress test elicited fetal heart rate decelerations. At planned cesarean section a viable male infant was delivered, with Apgar scores of 8 and 9 without meconium and an umbilical cord blood pH of 7.3. A characteristic feature of the sonogram photograph is the cloverleaf pattern of the true knot (Fig. 1). The diagnosis of a true knot in utero is possible. Prenatal management of such a finding should follow current established obstetric methods, which include fetal profile studies of Manning et al.1 and the fetal movement studies of Moore and Piacquadio.2

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The effect of amnioinfusion on uterine pressure and activity

To the Editors: I read with some misgiving the article by Posner et al. (Posner MD, Ballagh SA, Paul RH. The effect of amnioinfusion on uterine pressure and activ-

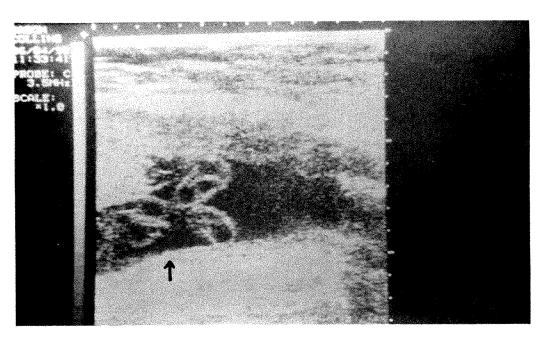


Fig. 1. Cloverleaf pattern of a true knot shown by arrow.

ity: A preliminary report. Am J OBSTET GYNECOL 1990;163:813-8). The authors noted a significant increase in uterine tone caused by amnioinfusion in 10 patients. In their Fig. 5 the uterine resting tone increased from 3 mm Hg to 33 mm Hg about ½ to 1 minute after the start of amnioinfusion; Posner et al. assumed a cause-and effect relationship. They ignored an increase in oxytocin (Pitocin) augmentation 6 minutes previously. Isn't the more logical cause simple oxytocin hyperstimulation? It would seem wise to exclude patients who are receiving oxytocin augmentation so that the uterotonic effects of oxytocin would not be confused with those of amnioinfusion.

Fred S. Miyazaki, MD

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Reply

To the Editors: We thank Miyazaki for his interest in our article. As he points out, oxytocin was infusing before the amnioinfusion. However, the dosage was not increased immediately before the beginning of the amnioinfusion; the dosage was simply written on the monitor strip at that time.

As part of our protocol oxytocin was allowed to be infused, but the dosage was not increased for a minimum of 60 minutes before the amnioinfusion. We included this as part of our protocol to control for the potential problem of uterine hyperstimulation. Because each patient was monitored for a 20-minute "steady-state" period before the amnioinfusion, this time period also included maintaining the oxytocin infusion at a constant dosage to prevent potential confounding clinical uterine hypersimulation.

We therefore believe that this event was real; even without including this patient in our calculations our results show a statistically significant increased uterine tone during and after amnioinfusion. We have been unable to identify those patients at risk for a hypertonic event; however, we continue to look into the problem.

Marvin D. Posner, MD

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Cesareans are more than skin deep

To the Editors: Use of the Allis test as proposed by Finan et al. (Finan MA, Mastrogiannis DS, Spellacy WN. The Allis test for easy cesarean delivery. Am J OBSTET GYNECOL 1991;164:772-5) to assure a skin incision that is approximately 15 cm in length will often result in an incision longer than that actually needed; even worse, the Allis test is no guarantee of an easy cesarean delivery.

The report does not mention having an adequate uterine incision and the techniques of assuring same. It also does not discuss differences found at the time of a primary cesarean as compared with the scarring and concretelike tissues that may be found in repeat

adhesions and adherent bowel loops. A vertical incision may be easily extended; however, the futility of extending the skin incision of a Pfannenstiel incision and the utility of cutting the muscles or detaching them from their insertion to obtain greater exposure when needed are not discussed.

A novice reading the report would get false assurance that a skin incision 15 cm long will assure easy cesarean delivery (when that is not the case) and would learn none of the measures that must be implemented when the predicted easy cesarean delivery becomes oh so difficult.

I also wonder how so many women still manage to deliver vaginally successfully when the hiatus in question is <15 cm. Perhaps adding advice about skills in addition to size would be a better predictor of easy cesarean delivery.

Sylvain Fribourg, MD

Letters 1899

13652 Cantara St., Panorama City, CA 91402

Reply

To the Editors: We thank Fribourg for the comments regarding our manuscript. The Allis test was not intended to be a gauge for skin incision length. As shown in Fig. 1 of our article, the Allis test is an objective means of measuring the distensibility of the entire anterior abdominal wall opening.

The size of the uterine incision was not measured in our report. Because we frequently extend the uterine incision with the operator's fingers, we have not found uterine incision size to be a frequent cause of dystocia at cesarean delivery.

The Allis test does objectively measure the distensibility of the skin incision, muscular defect, and fascial opening; thus it is a rough gauge of distensibility. It would indirectly reflect the concretelike tissues that Fribourg describes. The less elastic the tissue, the greater the incision size would have to be to pass the Allis test.

We have not found intraperitoneal adhesions and adherent bowel loops to be a cause of dystocia at cesarean delivery. These factors may occasionally cause difficulty with exposure. In our experience, as soon as the lower uterine segment is exposed, these factors do not restrict delivery. Because the objective gauge of delivery was the number of seconds from *uterine incision* to delivery, adhesions or adherent bowel loops would not have lengthened this time.

A grade IV difficult delivery was defined as "extension of the incision or muscle splitting" required to effect delivery. Incision of the rectus muscles in a transverse fashion (Maylard) may be required in a grade IV delivery. Detaching the rectus muscle from its insertion in the symphysis would certainly be another means of accomplishing this.

Finally, the question about successfully delivering vaginally when the cervix and vagina are not 15 cm dilated is a moot one. We doubt that the surgeons want a 1-hour second stage at cesarean section!

Michael A. Finan, MD

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, H. Lee Moffitt Cancer Center, P.O. Box 280179, Tampa,

Method of contraception	Typical pregnancy rate per 1000	Proportion of pregnancies that were ectopic	Typical ectopic rate per 1000	Rank with "typical" rates	Rank in Franks et a
Oral	30	0.0125	0.375	6	7).
Vasectomy	1.5	0.0125	0.019	7	7 } ti
Condom '	120	0.0125	1.500	3	5 '
Diaphragm	. 180	0.0125	2.250	2	4
Tubal sterilization	4	0.159	0.636	5	3
IUD	30	0.040	1.200	4 ·	2
None	520	0.0125	6.50	1	l

Table I. Ectopic pregnancy ranking of contraceptive methods: Circa 1980 to 1985

Alternative estimates of ectopic pregnancy risks during contraception

To the Editors: Franks et al. (Franks AL, Beral V, Cates W Jr, Hogue CJR. Contraception and ectopic pregnancy risk. Am J Obstet Gynecol 1990;163:1120-3) state that "differences in magnitude of ectopic pregnancy risk between contraceptives are so large that we believe the relative ranking of risk to be correct." The "typical" pregnancy rates of Trussell et al.¹ produce a different ranking of risk, however, for the United States in the early 1980s, as is shown in Table I. Conditional probabilities in Table I for U.S. copper intrauterine contraceptive devices (IUDs) are taken from Sivin and Stern,² and the proportion of pregnancies that were ectopic in the early 1980s are taken from Atrash.³

The typical pregnancy rates are for the first year of contraceptive use. Their division by a factor of three would bring the rates down to levels experienced by long-term users and also close to the values given as the lowest expected but would not change the rank order of the typical ectopic pregnancy rate.

Women using steroid-releasing IUDs have distinctly higher proportions of ectopic pregnancy (16% to 28%⁴) than the 4% found in copper IUD users² and have total ectopic pregnancy risks that overlap those of women who use no contraception (3.2 to 8.4 per 1000).

Very low risks of ectopic pregnancy among users of combined oral contraceptives may have increased since the early 1970s because of recent reformulations represented by triphasics and low estrogen dose.

Last, Franks et al. have not acknowledged prior similar approaches to estimation of ectopic pregnancy rates for women who use oral contraceptives, tubal sterilization, or IUDs, and for women who do not use contraceptive devices. Indeed, the same Hutterite fertility data cited by Franks et al. also served to estimate ectopic pregnancy rates for U.S. women not using contraceptive devices circa 1977 and for the period 1972 to 1976. The estimate of 2.6 ectopic pregnancies per 1000 U.S. women not using contraceptive devices in the early 1970s by Franks et al. is 90% of the value published in 1989 by Sivin. Virtually identical approaches yield virtually identical estimates of ectopic pregnancy rates in the United States in the early 1970s.

Irving Sivin, MA

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Reply

To the Editors: We appreciate Sivin's letter regarding our article. We find his calculations reassuring because they illustrate the utility of the method with a wide variety of assumptions. In spite of his using estimates for different time periods, a different type of contraceptive failure rate, and data from copper-containing IUDs rather than unmedicated IUDs, his results agree with our conclusions—that the use of no contraception carries the highest risk of ectopic pregnancy and that the use of oral contraceptives and vasectomy carry the lowest risk of ectopic pregnancy. We are pleased that his estimate of the rate of ectopic pregnancy for users of the newer copper-containing IUDs has a lower ranking of risk than we had calculated for the older, non-medicated IUDs.

We share Sivin's concern that risk of ectopic pregnancy may change when contraceptive methods are modified. It is imperative that accurate information be gathered on new contraceptives and on old ones that are reformulated. We believe that the approach we propose for estimating ectopic pregnancy risk will remain useful as new information becomes available.

Adele L. Franks, MD, Valerie Beral, MB, BS, Willard Cates, Jr., MD, MPH, and Carol J.R. Hogue, PhD Mailstop K30, OSA CCDPHP, Centers for Disease Control, 1600 Clifton Road, Atlanta, GA 30333

First report: Prenatal diagnosis of long cord

To the Editors: Leonardo da Vinci observed that the umbilical cord is as long as the fetus at any given age. This is reasonably correct at >10 weeks' gestational age; by 28 weeks' gestation the umbilical cord length is essentially achieved. Cord growth continues until term but is minimized by 32 weeks. Correlations to umbilical cord length include fetal movement, nutritional status, and congenital anomalies. Unfortunately no reports of umbilical cord length could be located as determined by prenatal ultrasonography.2 This seems important for no better reason than the relationship between cord length and umbilical cord complications. A long cord is predisposed to a true knot formation and nuchal cord formation.3 Because of this it seems reasonable to measure cord length, and 28 weeks' gestation seems a reasonable time to do so. With an ultrasonography unit (GE 3200, GE Medical Systems, Milwaukee) 30 pregnancies were screened at 28 weeks specifically for long cord formation. With the trace mechanism the cord was

followed as closely as possible and length estimated. A cord >50 cm at 28 weeks was identified in 1 of the 30 fetuses. A long cord measuring 100 cm was confirmed at delivery. It may not be necessary to have an exact length measurement at term. The 28-week measurement may be the best possible estimate. Once these fetuses are identified, watching them for umbilical cord complications may be worthwhile.

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Books received

- The A-Z of Women's Health. Edited by Derek Llewellyn-Jones. 260 pages, illustrated. New York, 1991, Oxford University Press. \$14.95.
- Anesthetic and Obstetric Management of High-Risk Pregnancy. Edited by Sanjay Datta. 719 pages. St. Louis, 1991, Mosby-Year Book, Inc. \$79.
- Antenatal Diagnosis of Fetal Abnormalities. Edited by J.O. Drife and D. Donnai. 363 pages. London, 1991, Springer-Verlag. No price listed.
- **Born Too Soon.** Edited by Elizabeth Mehren. 289 pages. New York, 1991, Doubleday. \$20.
- Breastfeeding: A Special Relationship. Edited by Mary Rose Tully and Mary L. Overfield. Videotape. Playing time 24 minutes. Raleigh, North Carolina, 1991, Eagle Video Productions. No price listed.
- Case Presentations in Obstetrics and Gynecology. Edited by T. Ian Wagstaff. 227 pages. Jordan Hill, Oxford, 1991, Butterworth-Heinemann. \$42.
- Chromosome Anomalies and Prenatal Development:
 An Atlas. Edited by Dorothy Warburton, Julianne Byrne, and Nina Canki. 104 pages, illustrated. New York, 1991, Oxford University Press. \$85.
- Craniofacial Abnormalities and Clefts of the Lip, Alveolus and Palate. Edited by Gerhard Pfeifer. 490 pages, illustrated. New York, 1991, Thieme Medical Publishers. No price listed.
- Conquering Infertility. Revised Edition. A Guide for Couples. Edited by Stephen L. Corson. 258 pages, illustrated. New York, 1991, Prentice Hall Press. \$9.95.
- Contraception and Mechanisms of Endometrial Bleeding. Edited by C. D'Arcangues, I.S. Fraser, J.R. Newton, and V. Oblind. 547 pages, illustrated. New York, 1991, Cambridge University Press. \$130 (soft cover).
- Current Obstetric & Gynecologic Diagnosis & Treatment. Seventh edition. Edited by Martin L. Pernoll. 1179 pages, illustrated. Norwalk, Connecticut, 1991, Appleton & Lange. \$37.95.

- The Doctors Book of Home Remedies. Edited by the Editors of Prevention Magazine Health Books. 738 pages. New York, 1991, Bantam Books. \$6.99.
- Schaffer and Avery's Diseases of the Newborn. Sixth edition. Edited by H. William Taeusch, Roberta A. Ballard, and Mary Ellen Avery. 1136 pages, illustrated. Philadelphia, 1991, W.B. Saunders Company. \$125.
- Everything You Need to Know About Prozac. Edited by Jeffrey M. Jonas and Ron Schaumburg. 154 pages. New York, 1991, Bantam Books. \$4.99.
- Genital Papillomaviruses and Related Neoplasms. Edited by Christopher P. Crum and Gerard J. Nuovo. 230 pages, illustrated. New York, 1991, Raven Press. \$82.
- Guide Pratique de Doppler en Obstetrique. Edited by Michele Uzan, Evelyne Cynober, and Catherine Benard. 108 pages, illustrated. Paris, 1991, Masson Editeur. No price listed.
- Handbook of Psychiatric Drug Therapy. Second edition. Edited by George W. Arana and Steven E. Hyman. 198 pages, illustrated. Boston, 1991, Little, Brown & Company. \$22.50.
- Hormone Toxicity in the Newborn: Monographs on Endocrinology. Edited by Fred A. Kincl. 334 pages. New York, 1990, Springer-Verlag. \$145.
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- Infertility and Reproductive Medicine, Clinics of North America: Workup of the Infertile Woman. Volume 2. Number 2. Edited by Michael P. Diamond, Alan H. DeCherney, and David Barad. 453 pages, illustrated. Philadelphia, 1991, W.B. Saunders Company. No price listed.

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- Menopause and Mid-Life. Edited by Robert G. Wells and Mary C. Wells. 161 pages. Wheaton, Illinois, 1991, Tyndale House Publishers. No price listed.
- Modern Approaches to Endometriosis. Edited by Eric Thomas and John Rock. 302 pages, illustrated. London, 1991, Kluwer Academic Publishers. \$87.50 (U.S.).
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- Nursing Care of the Childbearing Family: Study Guide. Edited by Laurie N. Sherwen, Mary Ann Scoloveno, and Carol T. Weingarten. 267 pages. Norwalk, Connecticut, 1991, Appleton & Lange. No price listed.
- OB/GYN Secrets: Questions You Will Be Asked. Edited by Helen L. Frederickson and Louise Wilkins-Haug. 308 pages. Philadelphia, 1991, Hanley & Belfus Inc. No price listed.
- Overcoming Hypertension. Edited by Kenneth H. Cooper. 454 pages. New York, 1991, Bantam Books, \$5.99.
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- Perinatal Anesthesia and Critical Care. Edited by James H. Diaz. 416 pages, illustrated. Philadelphia, 1991, W.B. Saunders Company. \$45.
- Period: Revised and Updated with a Removable Parents' Guide. Edited by JoAnn Gardner-Louklan, Bonnie Lopez, and Marcia Quackenbush. 87 pages, illustrated. Volcano, California, 1991, Volcano Press. \$9.95.
- Super Nutrition for Women: A Food-Wise Guide for Health, Beauty, Energy and Immunity. Edited by Ann Louise Gittleman and J. Lynne Dodson, 256 pages. New York, 1991, Bantam Books. \$10.
- Ultrasonography of the Urinary Tract. Third edition.
 Edited by Martin I. Resnick and Matthew D.
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 Williams & Wilkins. \$95.

Correction

In the article by Salafia et al. entitled "Placental pathologic findings in preterm birth" (AM J OBSTET GYNECOL 1991;165:934-8), in the list of authors on page 934, "C.A. Vogel, MD" and "K.F. Bantham, MD" should have been "C.A. Vogel, BA" and "K.F. Bantham, BA."

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby—Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

- Division of Neonatal Medicine, University of California Irvine Medical Center, "The New Decade: Neonatal/Perinatal Medicine," Alaskan Cruise Conference, September 8-15, 1992. For further information contact William G. Cvetnic, MD, or Terry Pliska, RN, Division of Neonatal Medicine, University of California Irvine Medical Center, P.O. Box 8119, Orange, CA 92664-8119. Tel.: (714) 634-6933.
- Common Problems in Obstetric Care II, April 20-25, 1992, Westin Maui, Maui, Hawaii. Sponsored by the Departments of Obstetrics and Gynecology, Cornell University Medical College—The New York Hospital and The University of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3059.
- Issues in Obstetrics and Gynecology, February 3-7, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.
- 4th Annual Review Course in Obstetrics and Gynae-cology, May 6-9, 1992, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA Category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.
- Third International Conference on Sound and Vibration in Pregnancy: Pregnant Women at Work, to be held February 7, 1992, in Gainesville, Florida. For information contact: Robert M.

Abrams, PhD, University of Florida, Department of Obstetrics and Gynecology, Box J-294, JHMHC, Gainesville, FL 32610. Tel.: (904) 392-3179.

- The International Symposium on "Diabetes and Pregnancy in the 90's" will take place in Tel Aviv, Israel, March 30-April 3, 1992. Main topics of the symposium will be gestational and preexisting diabetes in pregnancy. A special session in memory of Norbert Freinkel is scheduled. Abstract deadline is Dec. 31, 1991. Interested participants are requested to contact Dr. E. Shafrir, Chairman, or Dr. M. Hod, the Secretary, at the Secretariat, "Diabetes and Pregnancy in the 90's," P.O. Box 50006, Tel Aviv 61500, Israel. Fax: 972 3 655674.
- "Modern Concepts in the Management of Endometriosis," to air on Lifetime Medical TV. Important new information for primary care physicians and OB/GYNs on the diagnosis and treatment of endometriosis will be presented on Milestones In Medicine, a program which airs on Lifetime Medical Television and American Medical Television. Featuring guest host Robert L. Barbieri, MD, the program will examine the clinical basis for the emerging role of GnRH agonists and other modern therapies in the management of this often painful and debilitating disease. The program, made possible by an educational grant from Syntex Laboratories, Inc., is scheduled to air: every Sunday in December on Lifetime Medical TV at 6:30 PM ET/PT; on American Medical Television (on the Discovery Channel), on Dec. 15 and 22 at 10 AM ET, 9 AM PT; Dec. 12 and 26 at 2:30 PM ET, 11:30 AM PT, on Health & Sciences Network.

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- The relationship between umbilical artery Doppler velocimetry and fetal biometry (Scorza et al). 1991;165: 1013-9

Umbilical arteries, animal

Fetal hypertension induced by norepinephrine infusion and umbilical artery flow velocity waveforms in fetal sheep (van Huisseling et al). 1991;165:450-5

Umbilical cord

- Fetal hiccups and the umbilical ring (Collins). 1991; 165:1161 (Letter)
- First report: prenatal diagnosis of a true knot (Collins). 1991;165:1898 (Letter)
- First report: prenatal diagnosis of long cord (Collins). 1991;165:1900-1 (Letter)
- Funic reduction for the management of umbilical cord prolapse (Barrett). 1991;165:654-7
- Giacomello's observation and nuchal cords (Collins) (Letter); (Giacomello) (Reply). 1991;165:1895
- Relationship between fetal biophysical activities and umbilical cord blood gas values (Vintzileos et al). 1991;165:707-

Umbilical veins

An endothelial cell model for the investigation of the molecular regulation of fetal vascular tone (Dudley et al). 1991;165:1723-6

Urinary incontinence

Assessment of Kegel pelvic muscle exercise performance after brief verbal instruction (Bump et al). 1991;165:322-9

Urinary proteins; see Proteins

Urinary tract infections

- Effectiveness of antibiotic prophylaxis in preventing bacteriuria after multichannel urodynamic investigations: a blind, randomized study in 124 female patients (Baker et al). 1991;165:679-81
- Renal pelvicalyceal dilation in antepartum pyelonephritis: ultrasonographic findings (Twickler et al). 1991;165: 1115-9

Urine

Oligohydramnios: antepartum fetal urine production and intrapartum fetal distress (Groome et al). 1991;165:1077-80

Uterine activity; see Uterine contraction

Uterine cervix; see Cervix uteri

Uterine contraction

- The effect of amnioinfusion on uterine pressure and activity (Miyazaki) (Letter); (Posner) (Reply). 1991;165: 1898-9
- Effects of magnesium and terbutaline on contractility and K⁺ uptake in isolated human uterine muscle (Skajaa et al). 1991;165:1543-51
- Extended longitudinal study of uterine activity among lowrisk women (Main et al). 1991;165:1317-22
- Fetal movement during labor (Reddy et al). 1991;165:1073-6
- Home monitoring of uterine contractility (Rhoads et al). 1991;165:2-6 (Clin. opinion)

Uterine contraction-cont'd

Multicenter randomized clinical trial of home uterine activity monitoring for detection of preterm labor (Mou et al). 1991;165:858-66

Patterns of uterine activity after intravaginal prostaglandin E₂ during preinduction cervical ripening (Miller et al). 1991;165:1006-9

Qualitative evaluation of uterine contractions recorded by a double guard-ring tocodynamometer (Shinmoto et al). 1991;165:1282-6

Uterine activity after preterm premature rupture of the membranes (Campbell et al). 1991;165:422-5

Uterine contraction, animal

Circadian myometrial and endocrine rhythms in the pregnant rhesus macaque: effects of constant light and timed melatonin infusion (Matsumoto et al). 1991;165:1777-84

Forward shift in the initiation of the nocturnal estradiol surge in the pregnant baboon: Is this the genesis of labor? (Wilson et al). 1991;165:1487-98

Inhibition of oxytocin-induced uterine contractions by an oxytocin antagonist in the pregnant baboon (Wilson et al). 1991;165:456-60

Uterine neoplasms

Effects of peritoneal macrophages from patients with endometriosis on the proliferation of endometrial carcinoma cell line ECC-1 (Zhang et al). 1991;165: 1842-6

Endometrial morphology in asymptomatic postmenopausal women (Archer et al). 1991;165:317-22

Specific binding sites for insulin and insulin-like growth factor I in human endometrial cancer (Nagamani et al). 1991;165:1865-71

Ultrastructural study of glandular epithelium in adenomyosis in comparison with those of proliferative endometrium and well-differentiated endometrial cancer (Hayata). 1991;165:225-8 (Symposium)

Uterine rupture

Uterine rupture at term pregnancy with the use of intracervical prostaglandin E₂ gel for induction of labor (Maymon et al). 1991;165:368-70

Uterine rupture during trial of labor after previous cesarean section (Farmer et al). 1991;165:996-1001

Uterus, anatomy and histology

Histologic study of endometriosis and examination of lymphatic drainage in and from the uterus (Ueki). 1991;165:201-9 (Symposium)

Subserosal adenomyosis: a possible variant of pelvic endometriosis (Sakamoto). 1991;165:198-201 (Symposium)

Uterus, blood supply

Relaxation of the human uterine artery by atrial natriuretic peptide is independent of the endothelium (Bodelsson et al). 1991;165:483-4 (Letter)

V

Vagina, embryology

Renal agenesis in association with malformation of the female genital tract (Acién et al). 1991;165:1368-70

Vagina, microbiology

Bacterial vaginosis: current review with indications for asymptomatic therapy (Thomason et al). 1991;165:1210-

The vaginal ecosystem (Mårdh). 1991;165:1163-8

Vaginal birth; see Delivery

Vaginal birth after cesarean

External cephalic version after previous cesarean section (Flamm et al). 1991;165:370-2

The fetal-pelvic index: a method of identifying fetal-pelvic disproportion in women attempting vaginal birth after previous cesarean delivery (Thurnau et al). 1991; 165:353-8

Uterine rupture during trial of labor after previous cesarean section (Farmer et al). 1991;165:996-1001

Vaginal diseases

Adjunctive clindamycin therapy for preterm labor: results of a double-blind, placebo-controlled trial (McGregor et al). 1991;165:867-75

A new proline aminopeptidase assay for diagnosis of bacterial vaginosis (Schoonmaker et al). 1991;165: 737-42

Postpartum vaginal atrophy (Wisniewski and Wilkinson). 1991;165:1249-54

Thrombotic thrombocytopenic purpura first seen as massive vaginal necrosis (Gallup et al). 1991;165:413-5

The vaginal ecosystem (Mårdh). 1991;165:1163-8

Vaginal diseases, animal

Fungal morphology after treatment with itraconazole as a single oral dose in experimental vaginal candidosis in rats (Jansen et al). 1991;165:1552-7

Vaginal neoplasms

Laser vaporization of grade 3 vaginal intraepithelial neoplasia (Hoffman et al). 1991;165:1342-4

Primary invasive vaginal carcinoma (Eddy et al). 1991;165:292-8

Vaginal cancer: the role of infectious and environmental factors (Merino). 1991;165:1255-62

Vaginal smears

Adverse psychologic consequences of positive cytologic cervical screening (Lerman et al). 1991;165:658-62

Trichomoniasis: trends in diagnosis and management (Lossick and Kent). 1991;165:1217-22

Vaginitis

Bacterial vaginosis: current review with indications for asymptomatic therapy (Thomason et al). 1991;165:1210-7

Epidemiology of vaginitis (Kent). 1991;165:1168-76

A new proline aminopeptidase assay for diagnosis of bacterial vaginosis (Schoonmaker et al). 1991;165:737-42

Nonbarrier contraceptives and vaginitis and vaginosis (Roy). 1991;165:1240-4

The vaginal ecosystem (Mårdh). 1991;165:1163-8

Varicella-zoster virus

In utero diagnosis of congenital varicella zoster virus infection by chorionic villus sampling and polymerase chain reaction (Isada et al). 1991;165:1727-30

Vascular resistance, animal

Fetal hypertension induced by norepinephrine infusion and umbilical artery flow velocity waveforms in fetal sheep (van Huisseling et al). 1991;165:450-5

Vascular smooth muscle; see Muscle, smooth, vascular Vasculitis

Placental pathologic findings in preterm birth (Salafia et al). 1991;165:934-8. 1991;165:1948 (Correction)

Vasculitis, allergic cutaneous

Acute cutaneous vasculitis associated with prolonged intra-

venous ritodrine hydrochloride therapy (Bosnyak et al). 1991;165:427-8

Vasoconstriction

Endothelin-1-induced vasoconstriction is not mediated by thromboxane release and action in the human fetal-placental circulation (Myatt et al). 1991;165: 1717-22

Vasodilation, animal

Enhanced endothelium-derived relaxing factor activity in pregnant, spontaneously hypertensive rats (Ahokas et al). 1991;165:801-7

Vasodilator agents

Atrial natriuretic peptide: A vasodilator of the fetoplacental circulation? (Kingdom et al). 1991;165:791-800

Vasopressins

Inappropriate secretion of antidiuretic hormone in Sheehan's syndrome: a rare cause of postpartum hyponatremia (Putterman et al). 1991;165:1330-3

Oral-nasal membranes are not the major route for fetal absorption of amniotic fluid arginine vasopressin (Gilbert et al). 1991;165:1614-20

Vasopressin and operative hysteroscopy in the management of delayed postabortion and postpartum bleeding (Townsend et al). 1991;165:616-8

Vasopressin pack for treatment of bleeding after myoma resection (Townsend). 1991;165:1405-7

Venous pressure

Relationship between central venous pressure and pulmonary capillary wedge pressure in severely toxemic patients (Tellez). 1991;165:487 (Letter)

Venous thrombosis; see Thrombophlebitis Version, fetal

External cephalic version after previous cesarean section (Flamm et al). 1991;165:370-2

One-blade rotation of a persistent posterior vertex (Escamilla and Carlan). 1991;165:373-4

Vibration, animal

Effects of vibration frequency and tissue thickness on intrauterine sound levels in sheep (Richards et al). 1991;165:438-42

Vibroacoustic stimulation; see Acoustic stimulation Video display terminals; see Computer systems Videolaseroscopy; see Laser surgery

Villositis

Placental pathologic findings in preterm birth (Salafia et al). 1991;165:934-8. 1991;165:1948 (Correction)

Violence

An epidemic of antiabortion violence in the United States (Grimes et al). 1991;165:1263-8 (Clin. opinion)

Visual test; see Diagnostic tests

Vitamin E

The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood (Wang et al). 1991;165:1695-700

Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy (Wang et al). 1991;165:1690-4

Vulvar diseases

A case report of massive vulvar edema during tocolysis of preterm labor (Brittain et al). 1991;165:420-

Pudendal neuralgia (Turner and Marinoff). 1991;165: 1233-6

Vulvar vestibulitis syndrome: an overview (Marinoff and Turner). 1991;165:1228-33

Vulvar neoplasms

Vulvar. squamous cell carcinoma and papillomaviruses: Two separate entities? (Andersen et al). 1991;165:329-36

Vulvitis

Vulvitis and vulvovaginitis: cutaneous considerations (McKay), 1991;165:1176-82

Vulvovaginitis

Mycotic vulvovaginitis: a broad overview (Horowitz). 1991;165:1188-92

Vulvitis and vulvovaginitis: cutaneous considerations (McKay). 1991;165:1176-82

Vulvovaginitis: the role of patient compliance in treatment success (Nixon). 1991;165:1207-9

W

Water-electrolyte balance, animal

Fetal cardiovascular and fluid responses to maternal volume loading with lactated Ringer's or hypotonic solutions (Powers and Brace). 1991;165:1504-15

Weight lifting

Endocrine effects in female weight lifters who self-administer testosterone and anabolic steroids (Malarkey et al). 1991;165:1385-90

White blood cell count; see Blood cell count Wilson's disease; see Hepatolenticular degeneration Wood preservatives

Prolonged exposure to wood preservatives induces endocrine and immunologic disorders in women (Gerhard et al). 1991;165:487-8 (Letter)

World Health Organization

Psychosexual aspects of natural family planning as revealed in the World Health Organization multicenter trial of the ovulation method and the New Zealand continuation study (France). 1991;165:2077 (Abstract)

World Health Organization's policy considerations in natural family planning (Mehra). 1991;165:2075 (Abstract)

Wound healing, animal

Reduction of primary posttraumatic adhesion formation with the prostacyclin analog iloprost in a rodent model (Steinleitner et al). 1991;165:1817-20

Y

Yeasts

The in vitro activity of terconazole against yeasts: its topical long-acting therapeutic efficacy in experimental vaginal candidiasis in rats (Van Cutsem). 1991;165: 1200-6

Z

Zambia

Creating a demand for scientific natural family planning: the Zambian experience (Tafira). 1991;165:2070 (Abstract)

Evaluation of natural family planning programs in Liberia and Zambia (Kambic et al). 1991;165:2078 (Abstract)

Factors related to autonomy and discontinuation of use of natural family planning for women in Liberia and Zambia (Kambic and Gray). 1991;165:2060-2

Zambia—cont'd

Materials development in Zambia: concept versus reality (Tafira). 1991;165:2071 (Abstract)

Zona pellucida

Partial dissection of the zona pellucida of frozen-thawed

human embryos may enhance blastocyst hatching, implantation, and pregnancy rates (Tucker et al). 1991;165:341-5



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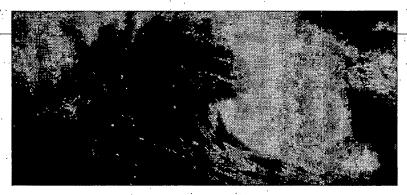
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FELLOWSHIP IN PELVIC SURGERY UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL

Department of Obstetrics and Gynecology University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

A comprehensive one-year training program in pelvic surgery is being offered by the Division of Pelvic Surgery in the Department of Obstetrics and Gynecology at UMDNJ-Robert Wood Johnson Medical School.

The program is designed to train fellows in all aspects of gynecological surgery including: Perioperative Care, Radical Surgery for Gynecologic Malignancies, Urodynamic Evaluation, Treatment of Incontinent Women, Vaginal Reconstructive Endoscopic Pelvic Surgery, Surgical Intensive Care Management, Management of Breast Disease.

The fellow will be actively involved in a number of clinical research projects and will receive instruction in the design of clinical investigations and data analysis. The position is ideally suited for individuals seeking a career in academic gynecology.

Interested candidates should send their curriculum vitae to: Nicholas Kadar, M.D., Director, Division of Pelvic Surgery, Department of Obstetrics & Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019.

The UMDNJ is an Affirmative Action/Equal Opportunity Employer M/F/H/V and a member of the University Health System of New Jersey.

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF CALIFORNIA, DAVIS

A full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento. Applicants should be Board eligible or certified in Obstetrics and Gynecology. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities.

Responsibilities of this position include inpatient and outpatient case management, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of applications is March 6, 1991.

The University of California, Davis is an equal opportunity, affirmative action employer.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO Obstetrics, Gynecology and Reproductive Sciences Assistant/Associate Professor Level Three Positions

The Department of Obstetrics, Gynecology and Reproductive Sciences, is seeking two full time faculty members at the Assistant or Associate Professor level. Applicants for these ladder rank positions should be board certified or eligible in Maternal-Fetal Medicine with primary activities in molecular and cellular perinatal biologic research and demonstrated capability of mounting an independent research program.

Send a curriculum vitae and bibliography to:

Robert N. Taylor, M.D., Ph.D.
Associate Professor
Department of Obstetrics, Gynecology and Reproductive
Sciences
University of California, San Francisco
San Francisco, CA 94143-0132

The Department of Obstetrics, Gynecology and Reproductive Sciences, is also seeking a full time faculty member at the Assistant or Associate Professor level to join the Maternal-Fetal Division at its San Francisco General Hospital campus. Applicants for this position should be board certified or eligible in Maternal-Fetal Medicine and should have excellent clinical skills and a demonstrated commitment to clinical or basic research and teaching. An interest in biophysical fetal assessment or infectious disease is highly desirable.

Send a curriculum vitae and bibliography to:

James R. Green, M.D.

Associate Clinical Professor
Room 6D-14, San Francisco General Hospital
1001 Potrero Avenue

San Francisco, CA 94110
UCSF is an Equal Opportunity/Affirmative Action Employer.
Women and minorities are encouraged to apply.

CHAIRPERSON DEPARTMENT OF GYNECOLOGY AND OBSTETRICS University of Kansas School of Medicine Kansas City, KS

Applications and nominations are being sought for the position of Chairperson for a dynamic University Department of 12 full-time clinical faculty, 4 full-time research faculty, and 12 residents. The Department is located at the Medical Center and fully integrated into the 474 bed University Hospital function. The candidate must have demonstrated leadership and organizational skills, be an effective clinician and teacher, and demonstrate expertise in scholarly activity. M.D. with Board certification in Obstetrics and Gynecology is required. Applications and nominations, including a curriculum vitae, should be sent to:

Laurence Y. Cheung, M.D.
Chairman, Obstetrics and Gynecology
Search Committee
Professor and Chairman, Department of Surgery
University of Kansas Medical Center
39th and Rainbow Blvd.
Kansas City, KS 66103

Candidates will be considered until the position is filled. The University of Kansas is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, NORTH CAROLINA

The Department of Obstetrics and Gynecology Division of Maternal and Fetal Medicine is seeking a perinatologist. In addition to prenatal diagnosis and other direct patient care activities, 50% of effort will be administrative responsibility for the clinical programs in a new ambulatory care center. Qualified individuals must have completed an ABOG approved fellowship, plus have experience in administration and proven ability to work with multidisciplinary teams. Salary dependent upon training and experience. Women and minorities are encouraged to apply. Please submit curriculum vitae and references to:

William Droegemueller, M.D., Chairman Department of Obstetrics and Gynecology UNC School of Medicine CB #7570, MacNider Bldg. Chapel Hill, NC 27599-7570

The University of North Carolina is an Affirmative Action/Equal Opportunity Employer.

FACULTY OPENINGS IN OBSTETRICS & GYNECOLOGY FOR GENERALISTS/PERINATOLOGISTS

Full time faculty positions at the University of South Alabama, Department of Obstetrics & Gynecology. Excellent opportunity for teaching, research and clinical service. Individuals must be board eligible or certified in Specialty/Sub-specialty. The academic rank and salary will be dependent upon the training and experience of the applicant. These positions are available immediately.

Please contact:

lan H. Thorneycroft, Ph.D., M.D.
Professor and Chairman
Department of Ob-Gyn
University of South Alabama
College of Medicine
2451 Fillingim Street
Mobile, Alabama 36617
(205) 470-5815

University of South Alabama is an Equal Opportunity Employer

The Department of OB/GYN at the Washington Hospital Center is currently seeking another full time Perinatologist for their Division of Maternal Fetal Medicine to work with the Director of Obstetrics/Maternal Fetal Medicine.

The Washington Hospital Center is a 900 bed tertiary care teaching facility with a compliment of 16 residents in the Department of OB/GYN. Currently the Department of OB/GYN delivers approximately 3,000 babies annually and because of its urban setting, the incidence of high risk pregnancies is approximately 40%.

There is an active Antepartum Surveillance unit and Prenatal diagnostic unit with OB/GYN ultrasound lab available in the division of Maternal Fetal Medicine. Invasive prenatal diagnostic procedures are performed in the division.

Excellent opportunity for active clinical research and resident teaching. We are interested in applicants who are qualified in Maternal Fetal Medicine, board eligible/board certified. Experience in OB/GYN Ultrasound and invasive prenatal diagnostic testing preferred.

Salary is commensurate with qualifications and experience.

Please send a Curriculum Vitae to Dr. Shailini Singh, Director, Obstetrics/Maternal Fetal Medicine, Washington Hospital Center, 110 Irving Street, NV, Suite 5W40, Washington, DC 20010 or Fax to 202/877-6590.



IRVINE—College of Medicine is seeking a candidate to occupy an Endowed Chair in Assisted Reproductive Technology at the Senior Professor level. The candidate should be a distinguished scholar with a background of exceptional research and have national and international standing. The candidate must also have outstanding teaching, clinical and administrative skills and have made and demonstrated potenial for original contributions to this field. Please send names and CV c/o Janet Nash, College of Medicine, Room 118, Med Surge 1, University of California, Irvine, Irvine, CA 92717. UCI is an Affirmative Action/Equal Opportunity Employer.



Wayne State University

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE HUTZEL HOSPITAL DETROIT MEDICAL CENTER

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FACULTY POSITIONS

FOR

DIRECTOR OF GENERAL GYNECOLOGY

THREE GENERALISTS IN OBSTETRICS AND GYNECOLOGY

The Department of Obstetrics and Gynecology at the Wayne State University School of Medicine is expanding rapidly and invites applications for the several faculty positions.

The candidate for director of General Gynecology should be an experienced leader and have an interest and a record in clinical research. The generalist candidates should be Board-eligible or certified. The positions involve clinical practice, graduate and undergraduate medical education, and clinical and/or laboratory research. Managed care experience is considered an asset. We offer an excellent opportunity for collaborative research with nationally recognized specialists. Academic rank and salary commensurate with experience and qualifications.

Please send CV and 3 letters of reference to: David B. Cotton, M.D., Professor, Chairman and Chief, Department of Obstetrics and Gynecology, Wayne State University School of Medicine/Hutzel Hospital, 4707 St. Antoine Blvd., Detroit, Michigan 48201, (313) 745-7283, FAX (313) 993-0689. Equal Opportunity Employer.



DIRECTOR MATERNAL FETAL MEDICINE

Department of Obstetrics & Gynecology University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

The Robert Wood Johnson Medical School invites applications for **Director of Maternal-Fetal Medicine**. The position of Director requires an individual with an established and broad understanding of medical education, research, patient care as well as effective administrative skills. Applicants should possess qualifications commensurate with those of a Professor of Obstetrics and Gynecology as defined by the Robert Wood Johnson Medical School.

The Division is located at St. Peter's Medical Center, the largest obstetrical and neonatal program in the State. Maternal-Fetal Medicine consists of five perinatologists, four sonographers, geneticist, two research nurses and one epidemiologist/biostatistician. The Robert Wood Johnson Program supports 7,000 deliveries per year.

Interested individuals should forward their curriculum vitae to: Robert A. Knuppel, M.D., M.P.H., Professor and Chairman, Department of Obstetrics & Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019.

The UMDNJ is an Affirmative Action/Equal Opportunity Employer M/F/H/V and a member of the University Health System of New Jersey.

Department of Obstetrics and Gynecology

University of California Irvine, College of Medicine. Obstetrics and Gynecology academic position: Associate Professor In-Residence/Professor In-Residence (M.D.), Director, Division of Gynecology, board certified. Extensive clinical experience in the management of ambulatory gynecology and gynecologic surgery. Previous administrative experience and special interest in infectious diseases or contraception desirable. Duties include medical student, resident, and postgraduate teaching, clinical service supervision, clinical and laboratory research. Private practice optional. UCI is an Equal Opportunity/Affirmative Action Employer. Send C.V. and five references to Thomas J. Garite, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, UCI Medical Center, 101 The City. Drive, Orange, CA 92668.

OB/GYN PRACTICE

Excellent opportunity for a board eligible physician to work in an established, two M.D. OB/GYN practice. Attractive salary plus bonus with equal partnership potential. Practice is located in beautiful Putnam County and is within an hour drive of Manhattan. Call Professional Practice Brokers at (516) 665-0439.

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Kevin Fiscella, M.D.

Medical Director

Geneva B. Scruggs Community

Health Care Center

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Buffalo, New York 14214



State University of New York Health Science Center Syracuse

MATERNAL-FETAL MEDICINE SUBSPECIALIST

The Department of Obstetrics and Gynecology of the SUNY Health Science Center at Syracuse is actively recruiting a fifth full-time Perinatologist for its Division of Maternal-Fetal Medicine.

The teaching hospital serves as the only Tertiary Care Center for the Regional Perinatal Program encompassing the 15 counties of Central New York with a population of 2.3 million.

Excellent opportunity to participate in teaching in a University based setting and collaborate in ongoing research in clinical Perinatology, Ultrasound, and Prenatal Diagnosis.

TOP SALARY, ATTRACTIVE BENEFITS PACKAGE, AND PROTECTED RESEARCH TIME.

Board eligibility/certification in Maternal-Fetal Medicine is required.

Interested candidates should send curriculum vitae to:

Robert K. Silverman, M.D.
Division of Maternal-Fetal Medicine
SUNY Health Science Center at Syracuse
Perinatal Center
725 Irving Avenue, Suite 115
Syracuse, NY 13210
(315) 470-7196

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Board certified or board eligible obstetrician/gynecologist to join two member department in multispecialty clinic. Office ultrasound, colposcopy, and cryosurgery available. Adjacent to 94 bed community hospital with special care nursery.

Worthington, MN is a community of 11,000 located in the highly productive corn belt. Recreational activities include good hunting, fishing, and two 18 hole golf courses. Excellent schools, pollution free environment, with low crime rate make this an ideal community for family living.

Excellent compensation package available. Respond to Mr. John Sieve, Administrator, Worthington Medical Center, 508 10th Street, Worthington, MN 56187 or call 507-372-2921.

PERINATOLOGIST

Perinatologist sought to develop a program of research in maternal-fetal medicine. The successful applicant will be primarily responsible for initiating research in both basic science and in clinical and epidemiologic aspects of maternal-fetal medicine in a university-based department of Obstetrics and Gynecology. The position is supported by two full-time faculty members in the division of maternal-fetal medicine that provide a strong foundation in clinical care and regional outreach. Deadline for applications is January 1, 1992.

Send C.V. to:

Search Committee c/o Millard Simmons, M.D. Chief, Division of Maternal-Fetal Medicine Department of Obstetrics and Gynecology West Virginia University, School of Medicine Health Sciences Center Morgantown, WV 26506

West Virginia University is an Equal Opportunity/Affirmative Action Institution.

FACULTY POSITION DIVISION DIRECTOR MATERNAL-FETAL MEDICINE

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The Department of Obstetrics & Gynecology at the University of Kansas School of Medicine-Wichita is accepting applications for the position of Director of the Division of Maternal-Fetal Medicine. This represents a unique opportunity to have the best features of both academic medicine and private practice.

Please send curriculum vitae to:

Daniel K. Roberts, M.D., Ph.D.
Professor & Chairman, Dept. of Ob/Gyn
University of Kansas School of Medicine-Wichita
HCA-Wesley Medical Center
550 N. Hillside

An Atlan

Wichita, KS 67214

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PHYSICIAN-IN-CHARGE OB/GYN EMERGENCY SERVICES

The Women and Infants Hospital of Rhode Island is seeking a candidate for the position of Physician-in-Charge Ob/Gyn Emergency Services. The job responsibilities include: patient care, teaching, research and assistance in divisional administration.

The minimum requirements are: active candidacy fcr, or certification by, the American Board of Obstetrics and Gynecology, qualification as an Assistant or Associate Professor of Obstetrics and Gynecology at Brown University, and proven teaching abilities. Rank and remuneration will be commensurate with experience and qualifications. If hired at the senior level the candidate must be board certified, have a national reputation and record of publication and research, along with teaching activities that are expected of a senior level position.

The Women and Infants Hospital of Rhode Island is an Equal Opportunity/Affirmative Action employer and actively solicits applications from minority and protected groups. Interested individuals should send an application and curriculum vitae to:

Patrick J. Sweeney, MD, PhD Chairman, Search Committee Women and Infants Hospital of RI 101 Dudley Street Providence, RI 02905

Applications are expected by January 1, 1992. Screening will begin on that date and will continue until a successful candidate has been identified or the search is closed.

MATERNAL/FETAL MEDICINE SPECIALIST

The Women and Infants Hospital of Rhode Island is seeking a candidate for the position of Maternal/Fetal Medicine Specialist. Responsibilities include: patient care (including prenatal diagnosis), teaching, research and administrative tasks.

Minimum requirements are: active candidacy for or certification in Ob/Gyn and Maternal Fetal Medicine by the American Board of Obstetrics and Gynecology. Qualification as an Assistant or Associate Professor of Obstetrics and Gynecology at Brown University, proven teaching abilities and an established research interest. Rank and remuneration will be commensurate with experience and qualifications. If hired at the senior level the candidate must be board certified, have a national reputation and record of publication and research, along with teaching activities that are expected of a senior level position.

The Women and Infants Hospital of Rhode Island is an Equal Opportunity/Affirmative Action employer and actively solicits applications from minority and protected groups. Interested individuals should send an application and curriculum vitae to:

Marshall Carpenter, MD Chairman, Search Committee Women and Infants Hospital of RI 101 Dudley Street Providence, RI 02905

Applications are expected by February 1, 1992. Screening will begin on that date and will continue until a successful candidate has been identified or the search is closed.

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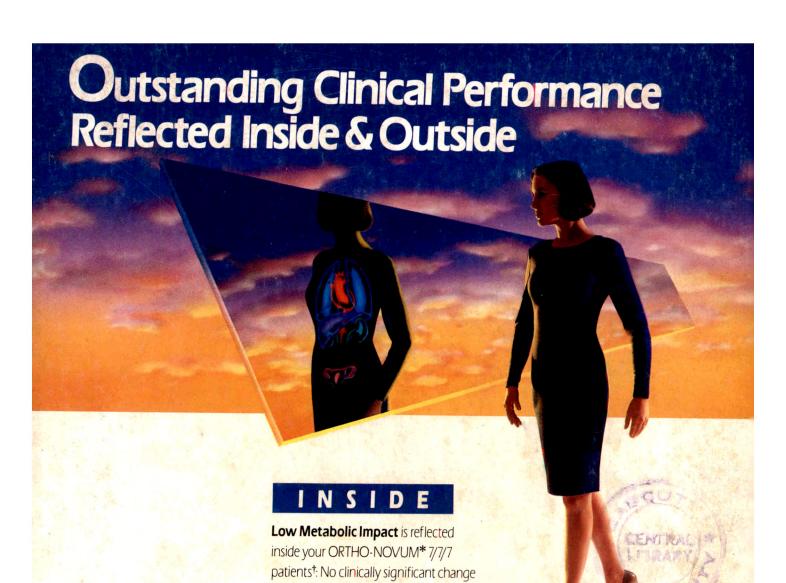
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Cigarette moking increases the risk of motivate cardiovasular size effects from varia contraceptives and the part of the part

that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4 HEPATIC NEOPLA-SIA Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptive. Oral contraceptives should be discontinued there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, appliedema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior US and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches shall not be permission users. SOULAR LESIONS There have been climical case reports of refinal introduciss associated with the use of oral contraceptives. Oral contraceptives should be described introducing the contraceptive or contraceptives or contraceptives or contraceptives or contraceptives or contraceptives or contraceptives or contraceptives. Permission of the contraceptive or contraceptives or contraceptive or contraceptives or contraceptive or contraceptives or should be understated in contraceptive or contraceptive or contraceptive sets of contraceptives or contraceptive or cont



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‡Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

\$An increase in blood pressure has been reported in women taking oral contraceptives.

Spellacy WN, Ellingson AB, Tsibris JCM.
Glucose and insulin levels after six months of reatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone.

J Reprod Med. 1989;34(8):540-542.

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December 1991 in two parts, part 2 volume 165, number 6

SUPPLEMENT TO

American Journal OBSTETRICS AND GYNECOLOGY

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NATURAL FAMILY PLANNING: CURRENT KNOWLEDGE AND NEW STRATEGIES FOR THE 1990s

Proceedings of a conference

Georgetown University Washington, D.C. December 10-14, 1990

Editors
John T. Queenan, MD
Victoria H. Jennings, PhD
Jeffrey M. Spieler, MSc
Helena von Hertzen, MD

Co-sponsored by the Georgetown University Institute for International Studies in Natural Family Planning, the United States Agency for International Development, and the World Health Organization



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American Journal of Obstetrics and Gynecology

Founded in 1920

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John T. Queenan, MD, and Kamran S. Moghissi, MD Washington, D.C., and Detroit, Michigan

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Introduction

A conference on "Natural Family Planning: Current Knowledge and New Strategies for the 1990s" was held at Georgetown University, Washington, D.C., on December 11-14, 1990. The conference was sponsored by the Institute for International Studies in Natural Family Planning, based in the Department of Obstetrics and Gynecology in the Georgetown University School of Medicine, and by the World Health Organization. Support was provided by the United States Agency for International Development through a cooperative agreement with the Georgetown Institute.

The purpose of the conference was to provide a forum for researchers, trainers, program managers, communicators, and educators to share the results of their work and chart new directions for increasing the knowledge, availability, and effectiveness of Natural Family Planning (NFP). Papers and discussion from 4 of the 13 conference sessions are included in this volume, along with abstracts of the remaining papers presented. The nine additional sessions will be published by Georgetown University in the spring of 1992.

NFP is an important topic for the fields of reproductive health and population. It is generally accepted that new hormonal or surgical methods of contraception will not be available in the next decade' and that the need for nonhormonal, noninvasive methods is well documented.2 Data from recent Demographic and Health Surveys indicate significant use of periodic abstinence—abstaining from intercourse for the purpose of avoiding pregnancy—in many countries throughout the world. However, most women who identify periodic abstinence as their family planning method have little knowledge of their fertility and thus use the method ineffectively. Many more who use no method of family planning or who would prefer to use natural methods are similarly uninformed. This leads us to ask such questions as:

- Can established NFP methods, such as the Billings ovulation method and the symptothermal method, be taught more efficiently? How effective are these methods in actual use?
- How can advances in prediction and detection of ovulation be applied to NFP? Will these advances

- make NFP easier to use? More effective? More acceptable to a wider variety of people?
- What are the outcomes of pregnancies that result from aged gametes (an issue of particular importance to NFP users, because pregnancies are most likely to occur from intercourse at the "extremes" of the fertile period)? Is the sex of the child determined by when intercourse occurs relative to ovulation?
- Given the effect of breastfeeding on fertility, how
 can an interface be created between breastfeeding
 and NFP? Can breastfeeding women use NFP?
 What role should the lactational amenorrhea
 method play in NFP? At what point postpartum
 do they need to begin to use NFP if they wish to
 avoid a pregnancy?

The current volume addresses these important questions. We believe the findings will help all of us address the fertility and reproductive health needs of women and couples in both developed and developing countries.

In addition, the conference responded to other important questions. Abstracts of these findings are contained in the current volume. The complete findings are to be published by Georgetown University in Spring 1992. It will include findings on such questions as:

- What are the behavioral issues that influence NFP use? How can abstinence during the fertile phase of the woman's menstrual cycle be dealt with in a variety of sociocultural settings?
- How have policies of funding agencies and other international organizations influenced the availability of NFP services? What steps can be taken to strengthen their support for natural methods?
- What are the best ways to provide fertility awareness information, which underlies NFP use, to special target audiences such as adolescents or low literacy women?
- How can NFP services be expanded while quality of services is maintained?
- How can various levels of health care providers be trained in NFP?

We hope our readers will find the current volume informative and thought provoking and that they also will take advantage of the additional information in the

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sessions to be published by Georgetown University in the Spring of 1992. We look forward to continued collaboration with our colleagues who participated in the conference and to a new dialogue with those whose interest in NFP is stimulated by the information presented here.

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Jeffrey Spieler, MSc Office of Population Bureau for Science and Technology Agency for International Development

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SESSION I. MAJOR SCIENTIFIC ISSUES IN NATURAL FAMILY PLANNING

John T. Queenan, Co-Chair Kamran S. Moghissi, Co-Chair Barbara Gross, Rapporteur

Natural family planning: Looking ahead

John T. Queenan, MD, and Kamran S. Moghissi, MD

Washington, D.C., and Detroit, Michigan

Natural family planning is used by a relatively small number of people worldwide. The Institute's goal is to increase the knowledge, availability, effectiveness, and acceptability of natural family planning. To do so, the Institute is conducting biomedical research in several areas including: (1) the development of a home test kit for cvulation prediction, (2) sperm-mucus interaction, (3) the mechanisms and fertility impact of lactational amenorrhea, and (4) outcome of pregnancies in natural family planning users. (AM J OBSTET GYNECOL 1991;165:1979-80.)

Key words: Natural family planning, ovulation prediction, sperm-mucus interaction, lactational amenorrhea, pregnancy outcome

When the Institute for International Studies in Natural Family Planning began its 5-year cooperative agreement with the Agency for International Development, numerous studies had identified the use-effectiveness of natural family planning (NFP) as 5 to 40 pregnancies per 100 women-years, roughly equivalent to the pregnancy rate for diaphragms (14%) and condoms (10%). The multicenter, multinational World Health Organization (WHO) study of the ovulation method of NFP demonstrated that for couples who used the method correctly, it was 97% effective for all sites combined. They also found that 94% of women could identify the fertile phase after a suitable training period of approximately 3 cycles.²

If a method can be so effective, why is it not more widely used? Although NFP use is relatively high among family planning users in some countries, such as Peru with 41%, the Philippines with 23%, and Haiti with 22%, its use in many other countries is far less. Considering all women, however, not just those who use a family planning method, the rate of NFP use is rarely as high as 5%.3

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20007. 6/0/33246 At the outset, our mission was to increase the knowledge, availability, effectiveness, and acceptability of NFP for child spacing worldwide. In 1987, breastfeeding as a means of child spacing also became a major focus of this project. While others in this conference are addressing issues of availability, effectiveness, and acceptability, we draw your attention here to issues of knowledge, specifically the information that responsible health care providers and clients need.

Current research

As we began our work at the Institute, it seemed obvious that to convince health professionals to support an increase in the use of NFP, we needed to concentrate on knowledge, in particular, on learning more about the aspects of reproductive physiology that affect NFP. Heretofore, most research focused on mucus and temperature changes as they delineated the fertile and infertile periods. Modern research techniques and recent scientific advances could offer possible new answers to several major questions. Thus our mandate was clear:

1. It was time to develop a simple home test kit to predict ovulation early enough to avoid pregnancy. This would be extremely useful in situations in which NFP is difficult to use effectively, such as during the perimenopause or in women with irregular cycles or vaginal infections. A home test kit could be even more important as an educational tool to reinforce how the body responds to the fertility cycle. It could help adolescents

- learn fertility awareness in a positive way as they develop self-confidence about their sexuality.
- 2. It was important to investigate not only the presence and quality of mucus but also what was happening at a molecular level to prevent and/or facilitate fertilization. This is important because it could identify the parameters of sperm entry and survival.
- 3. It was clear that lactation confers some degree of infertility. However, to recommend a method of child spacing relying on lactation-based parameters required the collaboration and consensus of the experts in the field, a requirement that was met by the Bellagio Consensus Conference on Breastfeeding (August 1988).4 As we study what regulates the gonadotrophins in lactation, we learn more about what differentiates the fertile and infertile periods and can speak more confidently about the specific parameters of lactational infertility and the lactational amenorrhea method.5 There is still concern about the safety of the lactation amenorrhea method, particularly regarding the health effects on the mother of promoting exclusive breastfeeding in borderline nutritional situations.6 Research on this and other maternal safety issues must be carried out to answer valid public health questions.
- Concern also remained about the safety of NFP, particularly with regard to pregnancy outcome.

Some scientists had suggested that pregnancies resulting from aging gametes, whether preovulation or postovulation, might result in suboptimal pregnancies. As with any method of family planning, it is important that we are able to provide information and make recommendations based on clear evidence of reproductive risk. The papers in this session address these issues.

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Fetal outcome among pregnancies in natural family planning acceptors: An international cohort study

Joe Leigh Simpson, MD,^a Ronald H. Gray, MD,^b John T. Queenan, MD,^c Robert T. Kambic, MSH,^d Alfredo Pérez, MD,^e Patricio Mena, MD,^f Michele Barbato, MD,^g Francisco Pardo, MD,^b Guillermo Tagliabue, MD,ⁱ Adenike Bitto,^b and Wilma Stevenson, BAⁱ

Memphis, Tennessee, Baltimore, Maryland, Washington, D.C., Santiago, Chile, Milan, Italy, Bogota, Colombia, and Lima, Peru

This international, multicenter, prospective cohort study examines the outcome of pregnancies associated with aging gametes. Comparing pregnancies conceived at or near the peak mucus phase with those occurring before or after the peak provides a means of evaluating the effect of aging gametes. The outcome criteria are (1) rates of spontaneous abortion. (2) low birth weight, and (3) congenital malformations. Preliminary analysis shows a trend toward increased spontaneous abortion with aging gametes in certain subsets (women with prior pregnancy losses) but no effect on birth weight. Too few method failures have yet been studied to make a definitive statement on congenital malformations. (AM J OBSTET GYNECOL 1991;165:1981-2.)

Key words: Natural family planning, fetal outcome, aging gametes, congenital malformations, spontaneous abortion

Fertilization involving aging gametes is associated with chromosomal abnormalities in animals, as reviewed elsewhere by our group. The phenomenon is thus plausibly said to be an inherent risk in inadvertent pregnancies occurring during the practice of natural family planning (NFP), a method based on abstinence during the fertile phase of the menstrual cycle. If an increase in chromosomal abnormalities result from aging gametes, one would expect an increase in spontaneous abortion rates, because at least half of all firsttrimester abortuses show chromosomal abnormalities. Unfortunately, methodologic shortcomings in assessing loss rates and anomaly rates in published studies of NFP users have precluded definitive statements concerning NFP safety despite the general belief that in the programmatic sense NFP does not pose undue risks. The best designed cohort study was that of the World Health

Organization; however, only pregnant women were identified, and many were lost to follow-up.²

Study design

In the current study reported here, two U.S. centers coordinate and analyze pregnancy outcome of couples who are tracked from acceptance in six recruiting centers in five countries (Chile, Peru, Colombia, United States, and Italy). When pregnancy is first recognized, the NFP chart is collected, to be assessed for likely date of conception by an independent panel of experts unaware of pregnancy outcome. Extensive intake information is gathered at the onset of pregnancy and again at 16 and 32 weeks. Prospectively following couples allows pregnancy losses to be identified in a timely fashion, minimizes "lost" cases, and allows potential confounding variables to be assessed. A systematic neonatal examination is performed, recording major and minor anomalies, birth weight, and other neonatal characteristics. Further details of our experimental design are provided elsewhere. The analysis compares pregnancy outcome in NFP user failure/planners versus NFP method failures, because theoretically only in the latter should pregnancies involving aging gametes occur. Specifically, we are assessing pregnancy outcome in relationship to the interval from estimated day of ovulation to conception date based on the times of intercourse and mucus peak. The latter has been shown to be the single most reproducible index of ovulation in NFP charts.3

Given the relatively low ratio of method failures to

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Supported by grant BR-US-004 from the U.S. Agency for International Development and the Institute for International Studies in Natural Family Planning, Georgetown University.

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user failures in our highly experienced recruiting center (1:4), a relatively large sample is necessary. This is especially true with respect to anomalies; small sample sizes or major inadequacies of previous studies. At present 766 pregnancies have been recruited from the six centers, with 706 completed and analyzed. Most subjects (90%) practiced symptothermal or mucus methods. Several independent observers (all experienced NFP providers) identified likely conception dates without knowledge of pregnancy outcome, as described earlier.³

Results

Preliminary data indicate that pregnancy loss (50/629 or 7.9%) is not increased in this NFP population (method failure, user failure, and planned pregnancies). However, a trend toward fewer pregnancy losses is observed among pregnancies conceived by intercourse ± 1 day of the mucus peak compared with those conceived by intercourse 2 or more days before the mucus peak (aging sperm) or 2 or more days after the mucus peak (aging oocytes). The trend reaches significance among women having pregnancy losses in previous pregnancies and is independent of maternal

age. Further analysis based on these and other conception intervals (e.g., day of the mucus peak and -1 as the optimal interval) will await expansion of our sample size.

Irrespective of any possible relationship between spontaneous abortion and timing of conception, the key health care issue relates to effects on liveborn infants. To this end, preliminary analyses showed that neither birth length, birth weight, nor head circumference was correlated with timing of conception. Rigorous assessment of the relationship between timing of conception and congenital anomalies in NFP will require a relatively larger sample to achieve requisite power, for which reason recruitment is still proceeding.

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Natural family planning and sex selection: Fact or fiction?

Ronald H. Gray, MD

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Determining sex of offspring by timing of intercourse has been a subject of intense interest for the lay public and professional community. This metaanalysis of couples practicing natural family planning provides an opportunity to evaluate sex ratios in relation to the timing of conception with several parameters, including basal body temperature shift and peak mucus as markers of ovulation. Data from six studies show a statistically significant lower proportion of male births among conceptions that occur during the most fertile time of the cycle. (AM J OBSTET GYNECOL 1991;165:1982-4.)

Key words: Natural family planning, sex selection, Y-bearing sperm, X-bearing sperm

There has long been interest in the relationship between sex ratio at birth and the timing of intercourse.

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Shettles¹ postulated that smaller Y-bearing sperm may have more motility in the midcycle cervical mucus and that intercourse around the time of ovulation would result in a predominance of male offspring. In 22 cases of midcycle conceptions, he reported 19 (86%) males, whereas with 19 conceptions more distant from ovulation, he observed 16 (84%) females.¹ Billings and Westmore² also reported more male infants with midcycle conceptions, and this has popularized the belief

Table I. Percentage of male births and relative risks of a male birth associated with timing of intercourse during the menstrual cycle

Study and definition of most fertile time	Male births	Female births	% Male	Relative risk of a male birth during the most fertile time relative to less fertile days (95% CI)
Guerrero ⁹	1			
Most fertile days*	139	168	45.3	0.82
Other	314	254	55.3	(0.71-0.95)
Harlap ¹⁶	319	327	49.4	0.92
Most fertile days† Other	1614	1399	53.6	(0.85-1.0)
WHO ⁷	15	11 .	57.7	0.96
Most fertile days‡ Other	44	29	60.3	(0.66-1.40)
Perez et al. ⁶		•		
Most fertile days‡	7	12	36.8	0.49
Other	25 .	8	75.8	(0.26-0.90)
France et al.5			•	,
Most fertile days‡	5	5	50.0	0.68
Other	17	6	73.9	(0.35-1.32)
Present study ⁸		•		
Most fertile days‡	86	87	49.8	1.06
Other	93	106	46.7	(0.86-1.31)
All studies				
Most fertile days	571	610	48.3	0.90
Other	2107	1802	53.9	(0.84-0.96)

WHO, World Health Organization.

that sex selection by timing of intercourse is feasible and successful. However, studies designed to test Shettles1 method have failed to confirm this finding.3,4 Despite this, natural family planning (NFP) programs in many countries promote natural methods to help couples select the sex of their child by timing of intercourse.

Study results

Several investigations allow an evaluation of the sex ratio associated with conceptions around time of ovulation compared with more distant conceptions. These studies are summarized in Table I. Four studies⁵⁻⁸ provide data on the day of conception relative to the mucus peak; one used basal body temperature shift,9 and one large study of Orthodox Jews from Jerusalem used menstrual cycle characteristics and days of sexual abstinence after menses to estimate probable conception day relative to ovulation.10 With these data, we estimated the percentage of male births and the relative risk of a male birth among conceptions during the most fertile time compared with conceptions at other times in the cycle. In all but one study, the proportion of male births and the relative risk of a male birth during the most fertile time were reduced, and overall, the relative risk was 0.90 (95% confidence interval 0.84 to 0.96), which is statistically significant. If the Jerusalem data are excluded and only NFP studies are considered, the relative risk is 0.86 (95% confidence interval 0.77 to 0.95). These results suggest that there is a small but statistically significant deficit of male births among conceptions during the most fertile phase of the cycle.

Table I shows the proportion of male births by estimated day of conception with data from NFP studies. The deficit of male births is associated mainly with conceptions during the 2 days before ovulation or on the estimated day of ovulation, and the excess of male births occurs among conceptions on days -3 and -4and days +2 and +3 relative to ovulation.

One possible explanation for such a deficit of male births is that high midcycle levels of gonadotrophin may favor X-bearing sperm. To test this hypotheses, James 11 examined the sex ratio after induction of ovulation in 27 studies. In this series of 2608 births, 46% were males, which is significantly lower than the expected sex ratio. In contrast, larger studies of ovulation induction with in vitro fertilization report a normal sex ratio of 51.7% male births.12,13 Thus high levels of gonadotrophin may

^{*}Most fertile days -1 or 0 from basal body temperature shift.

[†]Day of ovulation estimated from cycle length and abstinence after menses.

^{\$} Most fertile days -1 or 0 from mucus peak.

affect the sex ratio only if conception occurs in vivo, and this suggests that the hormonal milieu of the genital tract may be a determinant of the primary sex ratio.

Conclusion

In summary, the suggestion by Shettles and Billings that selection of male offspring by intercourse around the time of ovulation is possible is contradicted by scientific data. Although there is a statistically significant relative deficit of male births resulting from conceptions during the most fertile time of the cycle, this is insufficient to allow sex selection by timing of intercourse.

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Human cervical mucus: Research update

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Evaluation of cervical mucus is a standard for determining the fertile period in natural family planning. Cervical mucus accepts, filters, prepares, and releases sperm for successful transport to the egg and fertilization. Recent scientific advances provide answers to how the mucus regulates fertility as its physical properties change during the menstrual cycle. Transmission electron microscopy reveals small interstices between mucus macromolecules relative to a sperm head. Thus advancing sperm must push aside or cut through the microstructure. The interstices are largest in the periovulatory phase of the cycle. Small magnetic spheres, comparable with the size of a sperm head, are now being used to study the physical properties of the mucus on the scale of individual sperm. (AM J OBSTET GYNECOL 1991;165:1984-6.)

Key words: Natural family planning, cervical mucus, sperm, fertilization

The human cervix and its mucus complement have several roles in reproductive function. Of particular

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relevance to natural family planning (NFP) is their role in the modulation of sperm transport to the upper female reproductive tract. The concept of the fertile period, within which insemination can result in fertilization, is intimately linked to knowledge of cervix/mucus function. The cervix is the target organ of a sophisticated endocrine control system, one role of which is to modulate cervical mucus properties and secretion.

Thus the hormone signals are biomarkers, not just for ovulation per se, but for biologic mechanisms also responsible for fertility. The links between reproductive hormones and such mechanisms are only partially understood. The properties of cervical mucus are one of the primary links.

Function

Our direct knowledge of the properties and function of human cervical mucus derives primarily from in vitro studies. In vivo studies in ruminants and primates (both of which have a mucus-laden cervix) have suggested the validity of extrapolations to humans. Basic biologic and clinical aspects of the cervix, cervical mucus, and sperm have been addressed in a number of comprehensive reviews.1-3 We attribute four principal functions to cervical mucus in relation to sperm: (1) Sperm are admitted from the hostile vaginal environment; (2) the entering sperm are then filtered, and seminal plasma constituents are removed from their surfaces (only morphologically normal sperm are capable of proceeding through the mucus); (3) these sperm are nurtured and supported biochemically, perhaps to prepare the sperm for or to initiate capacitation (only after which the sperm can fertilize an egg); and (4) the cervical mucus is responsible for the storage of spermatozoa and their later release (this cervical reservoir function appears designed to coordinate the timing of insemination with that of ovulation).

Physicochemically, mucus is a gel, consisting of solid and liquid phases. The solid phase is a glycoprotein polymer (called mucin), whose macromolecules interact to form a network. Many aspects of how this network is created and maintained are not yet understood. For example, the existence and relative roles of molecular cross-linking and entanglement are not yet clear. The liquid phase of mucus contains soluble proteins, inorganic salts, enzymes, and other chemical constituents.

Structure

Our knowledge and ability to study mucus have benefited from improvement in and development of several experimental techniques. Table I summarizes some of the biophysical approaches that have been helpful. Our ability to perceive the topology of the cervical mucus microstructure has been facilitated by improvements in scanning electron microscopy4 and more recently transmission electron microscopy.5 These have revealed the heterogeneous, mosaic-like nature of the microstructure and its cyclic variations. The interstices between the mucus macromolecules are small compared with a sperm cell. Thus advancing sperm must push aside and/or cut through the microstructure.

Periovulatory mucus contains a more expanded microstructure with larger interstitial sizes than early pro-

Table I. New tools for studying cervical mucus and the sperm-mucus interaction

Transmission electron microscopy (freeze substitution) Topology of mucus microstructure Microrheometry (magnetic microrheometer) Physical (viscoelastic) properties of mucus on scale of individual sperm Laser light scattering Topology and physical properties of mucus microstructure Oher physical methods (nuclear magnetic resonance spectroscopy, flow permeation analysis, etc.) Properties of mucins per se, of mucus microstructure, and of water molecules within it Sperm motion analysis (computer-aided sperm analysis) Use of sperm as microprobe of mucus properties; simultaneous assessment of sperm motion and morphology Immunobeads Antibody-mediated sperm adherence to mucus microstructure Sperm image analysis Acrosomal status and other biologic properties

liferative or luteal-phase mucus. Indeed, initial physiochemical studies suggest that the principal determinant of cyclic changes in mucus is its water content or hydration. 6 Because of its high hydration, mucus begins to evaporate rapidly on removal from the cervix. Thus this property per se is not particularly amenable to laboratory analysis, much less so to the self-monitoring inherent in NFP. Qualitative biochemical or biophysical changes in the mucin microstructure are a more promising target for such monitoring.

Rheologic properties

The rheologic properties of whole mucus are viscoelastic and exhibit cyclic variation. In general, sperm penetration is maximal when viscoelasticity (i.e., the effective mucus viscosity and elasticity) is minimal, which tends to occur just before ovulation. However, the few careful studies of the cyclicity of such properties have demonstrated that there are significant variations both within, as well as among, normal women.7 More advanced analytic methods, which build on our improved understanding of the biology of the sperm-mucus interaction, would reduce the technical factors that contribute to such perceived variability.

A promising new physical approach for laboratory analysis is the use of microrheometry. In our laboratory, a small magnetic sphere, comparable in size with the sperm head, is forced through mucus by an electromagnet. The details of the relationship between such applied force and the movements of the particle provide information on the viscoelastic properties of the mucus. Earlier microrheometers used spheres 200 to 400 µm in diameter.7 By "titrating" the size of the sphere down to that of the sperm head, new information on the mucus properties experienced by an individual sperm can be obtained. Such analysis is now commencing in our laboratory and should provide a more incisive perspective of mucus receptivity to sperm.

It should be appreciated that the electrochemical interaction between the sperm surface and the mucus macromolecules can also contribute to the mucus' resistance to sperm.² Increases in such affinity may be responsible for the filtering of morphologically abnormal sperm by the mucus.⁸ Little is known about the details of this phenomenon, which may also be responsible for other alterations in sperm penetrability through mucus.

Penetrability

Sperm penetration into mucus is a phenomenon dependent jointly of sperm and mucus properties. "Penetrability" of mucus implies several features of the sperm-mucus interaction, including the number (or fraction) of sperm that cross the mucus interface and the rate at which such sperm subsequently migrate through the mucus interior. The distinguishing characteristics of sperm that can successfully penetrate (and escape from) mucus are not fully appreciated. Human semen specimens with more vigorous sperm have increased ability to penetrate ovulatory human mucus. Morphologically abnormal sperm (which have inferior motility when compared with morphologically normal cells¹¹) have great difficulty penetrating mucus. 8

The structure and physical properties of the mucus interface are different from those of the interior,⁵ generally offering greater resistance to sperm progression. Thus care must be taken in peforming analysis of mucus penetrability to control for the size and shape of the interface and for the quality of the semen applied to the mucus. The common configurations for such analysis are plane slide preparations or small capillary tubes, now of rectangular cross section.^{9, 12-14}

In general, the number and rate of penetration of sperm into mucus are maximal during the periovulatory period, when viscoelasticity is minimal. However, sperm can penetrate the cellular, more viscoelastic mucus that occurs before ovulation, earlier in the proliferative phase of the menstrual cycle. Preliminary data show a correlation between such precocious sperm penetrability and rising serum (or urinary) estrogen levels. Sperm penetrability begins to drop immediately after the peak in luteinizing hormone secretion.

It should be emphasized that in vitro sperm penetration into mucus does not guarantee sperm survival and escape from the mucus, both of which must occur in vivo. Understanding the sperm-mucus interaction must allow for changes in mucus properties in vivo after initial sperm entry. These considerations are particularly relevant to NFP, in which changes in mucus properties and/or the sperm-mucus interaction can contribute to the determination of the fertile interval during the menstrual cycle.

In conculsion, developments in our knowledge of mucus and sperm and in methods for their analysis can now be used to perform more integrated, sequential analysis of how mucus accepts, filters, prepares, and releases sperm for successful transport to the egg and fertilization.

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Symptothermal and hormonal markers of potential fertility in climacteric women

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One hundred seventy-seven menstrual cycles in 36 women between 45 and 53 years of age were studied prospectively. All the women were experienced in the symptothermal method of natural family planning. The objective was to determine the symptothermal and hormonal indices of potential fertility by measuring urinary estrone glucuronide and pregnanediol glucuronide. Thirty-three percent had regular cycles consistent with potential fertility, 19% had cycles consistent with infertility, and 47% had a mixture of both types of cycle. (AM J OBSTET GYNECOL 1991;165:1987-9.)

Key words: Natural family planning, climacteric, menopause, fertility

The climacteric has been defined as a gradual transitory phase in the human female between the ages of reproductive and nonreproductive ability. The time when fertility starts to decline is difficult to determine for an individual. The mean marital fertility rate, however, is markedly reduced in women who are over 45 years old. The menopause (i.e., the last menstrual period) occurs most frequently during the fiftieth year of life. This event is a sign that potential fertility has been lost, but the time can only be determined retrospectively (usually after 1 year has elapsed). Accordingly, there is a need for reliable methods to determine the times of potential fertility in women who are over 47 years old and premenopausal.

Use of natural family planning

Natural methods of family planning depend on the detection and interpretation of clinical signs of potential fertility. The results from studies of preclimacteric women (aged 25 to 35 years) have shown that symptothermal changes (e.g., in basal body temperature and the characteristics of cervicovaginal mucus) correlate well with indices of ovarian function and the probable times of potential fertility. In addition, the results of prospective clinical trials have shown that methods of contraception based on periodic abstinence can be as

effective as most alternative procedures.^{5, 6} During the climacteric, however, the traditional signs of potential fertility are frequently erratic and difficult to interpret.⁷⁻¹³ Furthermore, new signs of diminished fertility (e.g., hot flushes, night sweats, and mastalgia) start to appear, but their significance in natural family planning has not been evaluated. Consequently, we have undertaken a prospective, comprehensive study of symptothermal and hormonal indices of potential fertility during the climacteric.

Aims

There were five principal aims: (1) to determine the within- and between-women variation in menstrual cycle length during the climacteric; (2) to determine daily changes in the urinary excretion of hormone metabolites; (3) to define criteria for potential fertility; (4) to examine temporal relationships between the times of defined changes in clinical and hormonal indices of ovarian function; and (5) to ascertain the most reliable indicators of potential fertility during the climacteric.

Plan of investigation

We planned to study 40 women (aged 45 to 54 years), who were experienced users of the symptothermal method of natural family planning, for 6 calendar months. All volunteers were asked to complete a daily record of vaginal bleeding, basal body temperature, the characteristics of the cervix and cervicovaginal mucus, and climacteric symptoms (hot flushes, night sweats, breast tenderness, and dyspareunia) that occurred during the previous 24 hours. In addition, each woman collected a daily sample of early morning urine for retrospective analysis of estrone glucuronide (EG) and pregnanediol glucuronide (PG) by time-resolved theorescence immunoassay.

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Table I. Parameters for determining the rise (by CUSUM analysis) and peak days of urinary EG and PG

Analyte	Baseline	k	h	NCV	Peak	Limits
EG PG	(Days) 1 to 6 4 to 9	(Loge) 0.15 0.20	(Loge) 0.40 1.80	1 3	Confirm. 4 —	(Days) -18 to -9 >-20

Where: baseline is the set of days over which the mean of loge analyte values was calculated to give a base level for the CUSUM; k is the value added to the base level, and h is the CUSUM decision level; NCV is the number of consecutive values required to be above the reference value (base level + h); peak continuation is the number of subsequent lower values that must occur after the highest arithmetic value; limits refer to an acceptable interval between the peak EG or rise in GL and the end of the cycle (day 0).

Table II. Classification of menstrual cycles according to defined changes in the concentration of urinary EG and PG

		Presence of				
Analyte	Rise	Peak	In limits*	Class	No.	%
EG	Absent	Absent	_	0	22	12
	Absent	Present	_	1	11	6
	Present	Present	No	2	17	10
	Present	Present	Yes	3	127	72
PG	Absent	_	_	0	40	23
	Present	_	No	1	14	8
	Present	_	Yes	2	123	69

^{*}Interval from peak EG or rise of PG to day of next menses.

Table III. Cross tabulation of menstrual cycles by class according to changes in urinary EG and PG

EG class	0	1	2	Total
0	12	2	8	22
1	7	0	4	11
2	6	8	3	17
3	$\frac{15}{40}$	<u>4</u>	$\frac{108}{123}$	<u>127</u>
Total	40	14	123	177

Preliminary results

Thirty-six women (aged 45 to 53 years; median, $45\frac{1}{2}$ years) were recruited, and 177 menstrual cycles were identified retrospectively (length, 16 to 103 days; median, $27\frac{1}{2}$ days). The number of cycles per woman ranged from two to seven.

Potential fertility

Defined changes in the daily concentrations of EG and PG were used to classify each menstrual cycle retrospectively as potentially fertile or not potentially fertile (i.e. infertile). The parameters for the algorithms¹⁴ used to identify a rise and peak in the urinary excretion of EG and a rise in PG were derived from a second analysis of the data obtained from a similar study of 73 women (aged 21 to 35 years) over 118 menstrual or conception cycles.¹⁵ The values and additional criteria

to determine potential fertility for this study are shown in Table I.

The menstrual cycles during the climacteric were classified according to the changes in EG and PG as shown in Table II. A cross tabulation of the classification is shown in Table III. Sixty-one percent (108/177) of the menstrual cycles were defined as potentially fertile on the basis of criteria for EG and PG appearing normal. The numbers of all women (and menstrual cycles) showing the characteristics consistent with potential fertility, infertility, and a mixture of cycles that produced periods of potential fertility are shown in Table IV. Of the 88 cycles from women in group 3, 45 were potentially fertile and 43 were infertile.

Comment

The results from this preliminary analysis of the data suggest that about 33% of women between the ages of 45 and 54 years experienced regular menstrual cycles that were consistent with periods of potential fertility. About 19% had menstrual cycles without signs of potential fertility, and the remainder (47%) had a mixture of both types of cycle. This method of classification is likely to overestimate the number of potentially fertile cycles (because ovulation need not necessarily occur even in the presence of normal hormonal changes). This probability means that we will error on the side of caution when the results are used to develop a strategy for contraceptive practice.

The data are also consistent with epidemiologic ev-

	Potential	lly fertile	Infe	rtile	potentia	ture lly fertile ıfertile	To	ptal
	No.	%	No.	%	No.	%	No.	%
Women Cycles	12 63	33 36	7 26	19 15	17 88	47 50	36 177	100 100

Table IV. Number of all women (and menstrual cycles) classified according to potential fertility

idence of a gradual transition over time from regular cycles of potential fertility to total infertility. An analysis of the data from women with potentially fertile cycles has shown that the cycle length and clinical indicators have similar usefulness for predicting the start and finish of a defined fertile period to those observed in younger women.¹⁴ Conversely, a first analysis of our data has shown that the same clinical indicators may not always reflect the underlying physiology in women with infertile cycles (data not shown). These aspects will be a smaller proportion of the subjects of a subsequent publication. Consequently, it is apparent that new markers are required to predict those cycles in which a period of potential fertility will occur from those in which it will not. A simple dipstick for a urinary metabolite that provided this information would greatly reduce the false-positive rate of subsequent tests to define precisely the time of potential fertility.

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Breastfeeding: A natural method for child spacing

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Lactational amenorrhea plays an important role in child spacing. Recent research has led to a consensus regarding the status of lactational amenorrhea as a method of family planning. This is currently referred to as the lactational amenorrhea method. Research priorities were to field test the lactational amenorrhea method and define the factors that influence lactational amenorrhea and the interface between the lactational amenorrhea method and other family planning methods. (AM J OBSTET GYNECOL 1991; 165:1990-1.)

Key words: Lactational amenorrhea method, child spacing, breastfeeding, postpartum contraception, lactational amenorrhea

Areas of agreement

Although it has been known for a long time that breastfeeding causes a variable period of amenorrhea, it is only during the past 10 years that scientific attention has been focused on its role in fertility regulation.\(^1\) Progress in this area of research has led to general agreement in four main areas.\(^2\) First, it is now clear that breastfeeding is associated with a reduction in fertility, which is closely related to the duration of amenorrhea.\(^3\) This has been shown particularly in those populations in whom prolonged breastfeeding, in the absence of artificial contraception or postpartum sexual abstinence, causes substantial durations of infertility that may last for as long as 2 years or longer.\(^4\)

Second, detailed endocrine studies have shown that inhibition of ovulation through gonadotrophin suppression is a key factor in lactational amenorrhea.⁵ After the resumption of menstruation, breastfeeding may reduce fertility to some extent, but the effect is much less marked compared with the period of lactational amenorrhea.⁶

The third factor of importance is that the maintenance of lactational amenorrhea is suckling dependency.⁷ Those mothers who suckle frequently for long periods and maintain breastfeeding at night experience longer periods of lactational amenorrhea than those who reduce suckling frequency or duration at an early stage, sometimes in response to the rapid introduction of supplementary feeds.⁸ Because breastfeeding practices differ widely across cultures, it has proved impossible to define a minimum level of suckling that will

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guarantee the maintenance of lactational amenorrhea and that could be applied universally. Nevertheless, many studies have shown that more intensive suckling is associated with longer periods of lactational amenorrhea.²

The fourth point on which there is general agreement is that lactational amenorrhea makes an important contribution to overall fertility as expressed by completed family size, particularly in those countries in which the use of artificial contraception is low.9 At an individual level, lactational amenorrhea makes an important contribution to the effective spacing of births, which, in turn, has important advantages for both infant mortality and morbidity. All these factors strongly add to the justification for making the protection and promotion of breastfeeding an important international health objective.

Future research issues

Despite the progess made during the past 10 years, many important questions about the use of breastfeeding for child spacing remain unanswered. The Institute and others have initiated several studies in this area. The Bellagio Consensus statement² notes that during the first 6 months, postpartum mothers who fully or nearly fully breastfeed and maintain lactational amenorrhea can expect a pregnancy rate of less than 2%. An algorithm incorporating these three criteria (the infant is <6 months of age, the mother is amenorrheic, and full or really-full breastfeeding is being practiced) has been developed by Dr. Miriam Labbok and serves as the basis for the lactational amenorrhea method (LAM).10 It is important that the robustness of these guidelines be tested in field studies within a number of different cultural settings so that they can command the confidence of health workers advising postpartum mothers about contraception.

A second priority is to define more fully those factors that may influence the duration of lactational amenorrhea. Possible factors that may be of importance include maternal nutrition, racial differences, individual biologic variation, and other factors such as maternal illness. A multicenter study of 550 subjects in each of seven centers has been funded by the World Health Organization and supported by the Institute for International Studies in Natural Family Planning to define those confounding factors that are of principal importance in determining the duration of lactational amenorrhea.

The third area of importance for future study is the interface between LAM and alternative methods of family planning. Towards the end of lactational amenorrhea, fertile signs may be particularly difficult to interpret, and very little is known about what factors influence mothers and health professionals to determine when alternative family planning methods are required. The strategy of introducing artificial contraception at an early stage during the postpartum period will not in many cases be either necessary or an effective use of scarce resources.

The final priority for the future is to disseminate accurate and effective information about LAM that can be easily interpreted by health workers and understood by breastfeeding mothers. A major need exists for effective operational research to determine the best ways whereby the substantial advantages of LAM can be used to best effect in both developing and developed country

settings. However, it is clear that by ensuring effective birth spacing, breastfeeding and LAM will continue to be an important factor in both maternal and child health, particularly in developing countries.

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Conclusion

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Much biomedical research has been accomplished during these 5 years of our cooperative agreement, and considerable work is still in progress. As we look at the use of natural forms of fertility regulation for the future, definitive areas that need additional scientific study include the following:

- 1. Develop a simple home test kit that could predict ovulation 4 to 5 days in advance.
- Determine whether aging gametes, as seen in natural family planning (NFP) "method failures," have any bearing on incidence of spontaneous abortion, low birth weight, or congenital malformations.
- 3. Determine a definitive answer on whether aging gametes are related to sex selection.
- 4. Study mechanisms of mucus receptivities and hostility to sperm in promoting and preventing pregnancy to explore areas for modification of or improvement in periodic abstinence.
- Study the perimenopause and other special circumstances to elucidate how women in these reproductive states can use periodic abstinence effectively.
- 6. Study the lactational amenorrhea method (LAM) in a variety of clinical settings to test the assertion of 98% effectiveness for preventing pregnancies for the first 6 postpartum months.
- 7. Study the health sequelae of exclusive breast-feeding.

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- 8. Study the use-effectiveness of LAM beyond 6 months, beyond supplements to, and beyond menses return.
- 9. Study the neuroendocrinology of lactation with the goal of enhancing the infertile period.

Such studies require considerable financial support. The basic science studies often are funded by the National Institutes of Health or private research foundations. The use-effectiveness and some of the basic science studies have been funded by the Agency for International Development and the World Health Organization. A need still exists for additional study, and therefore considerably more funding. We hope that the information presented at this meeting will challenge and inspire a broad spectrum of organizations to support research in natural fertility regulation (NFP and LAM)—the international donor organizations, public and private research agencies and foundations, and religious groups whose beliefs proscribe the use of any methods other than the natural methods.

As each new family planning method is developed, it is only a matter of time until major and minor drawbacks are recognized. NFP is no exception. Certainly it has its drawbacks, including the requirements for self-observation and the need to adjust behavior to meet fertility intentions—in short, the drawbacks of any user-dependent family planning method. Because NFP uses no chemical or device, it will always be attractive to certain groups of women in their reproductive years. Because the cost of education and maintenance is negligible, NFP has a very practical importance from a public health standpoint. If couples are exposed to accurate data on this method, it will become an attractive alternative for many.

SESSION II. OVULATION PREDICTION: PRESENT DEVELOPMENTS AND FUTURE NEEDS

Michael J. Zinaman, Chair John T. France, Co-Chair Sanford Markham, Rapporteur

Overview and issues in ovulation prediction

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Recent advances in our understanding of the physiology of reproduction have led to the ready availability of tests that allow women to self-monitor their fertility potential. Home pregnancy tests were the first to be marketed to the general public. Shortly thereafter, urinary luteinizing hormone tests also became available. In the privacy of her own home, a woman can now comfortably monitor for impending ovulation; by the time of her expected menstrual period, she can identify whether or not she is pregnant. However, there remains a great need for the development of further tests that women can use to identify the days of her cycle on which sexual intercourse is likely to result in pregnancy. (AM J OBSTET GYNECOL 1991;165:1993.)

Key words: Reproductive physiology, self-monitoring, fertility potential

Advances in research have allowed us to show that sperm can survive in human cervical mucus and maintain their fertility potential for up to 5 days or perhaps even more. Much of their survival appears to be dependent on the presence and characteristics of the mucus itself. At the same time, we have recognized that the fertile period ends perhaps as early as 12 hours

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after ovulation. It has been a role of the Biomedical Division in the Institute to support research to develop simple tests for the self-monitoring of fertility potential including, in particular, the identification of both the onset and end of the fertile period. The use of such tests will allow couples to enhance their fertility awareness and increase their knowledge of their reproductive status.

The following papers present some of the recent progress made by both industry and academic researchers toward producing simple test systems that might be used in the home for defining the fertile period. It is apparent that while much work has been done, significantly more effort is needed to make these tests a reality.

The evolution of reference methods to monitor ovulation

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Reference methods to predict or detect ovulation have evolved substantially during the past few decades. The potential use and limitations of such methods are reviewed. The use of transvaginal ultrasonography with color flow mapping to monitor periovulatory changes in the intrafollicular morphology and blood flow is described. (AM J OBSTET GYNECOL 1991;165:1994-6.)

Key words: Ovulation prediction and detection, vaginal ultrasonography, oocyte, fertile period

Ovulation is the release of one or more oocytes from the ovarian follicle. The event is preceded and followed by a complex series of functional and morphologic changes within the ovary and other organs of the female reproductive tract. The only definitive evidence that ovulation has occurred is the identification of an ovum or preembryo in an extraovarian location or by the detection of a pregnancy. Neither of these endpoints, however, can be used to evaluate reference methods for the prediction or detection of ovulation. Inevitably, there will be oocytes that are released but not found before or after fertilization or do not result in a pregnancy. This limitation invalidates the calculation of the positive and negative predictive values for any method. Nevertheless, more practical reference methods have been developed to monitor the presumed time of ovulation with the aim of helping women achieve or avoid a pregnancy.

Evolution of reference methods

The principal methods that are evolving are based on the following: (1) calendar calculations^{1, 2}; (2) a defined rise in basal body temperature³; (3) the presence and type of cervical mucus⁴; (4) defined changes in the concentration of circulating hormones⁵; (5) the immunoassay of urinary steroid glucuronides and luteinizing hormone⁶; (6) changes in ovarian morphology^{7, 8}; and (7) changes in intrafollicular blood flow.^{9, 16}

Reference methods are often modified for self-use. An improved algorithm has been developed to determine the day of shift in basal body temperature, 11 and an electronic thermometer has been manufactured and evaluated. 12 A special syringe has been produced to sample and measure the volume of cervicovaginal fluid, 13 and a simple visual test has been devised to determine the activity of the peroxidases. 14 A major

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Table I. Time intervals (based on probit analysis) between ovulation and preceding changes in the immunoconcentration of plasma hormones

	Time (hr)		
Variable	Mean	Range	
Estradiol rise	83	48-168	
Estradiol peak	24	. 0-48	
LH rise	32	24-56	
LH peak	17	8-40	
Progesterone rise	· 8	0-32	

LH, Luteinizing hormone.

Table II. Time intervals between ovulation (based on follicular size and echogenicity) and preceding changes in the immunoconcentration of plasma hormones

•	Time (hr)		
Variable	Mean	Range	
Estradiol peak	·25	21-33	
LH rise	. 30	27-57	
LH peak	15°	9-45	
Progestrone rise	12	9-21	

LH, Luteinizing hormone.

study organized by the World Health Organization⁵ involved the use of a histologic endpoint to relate defined changes in the concentrations of circulating hormones to the time of ovulation. The results are summarized in Table I.

Immunoassays for urinary estrone glucuronide (a metabolite of plasma estradiol) and urinary pregnanediol-3α-glucuronide (a metabolite of plasma progesterone) have been simplified for self-use in the form of immunotubes, ¹⁵ and urinary luteinizing hormone can be measured on an immunostick. Transabdominal ultrasonography has also been used to study the time intervals between defined changes in the concentration of plasma hormones and the time of follicular rupture (ovulation). ¹⁶ The results are summarized in Table II.

Ovulation monitoring 1995

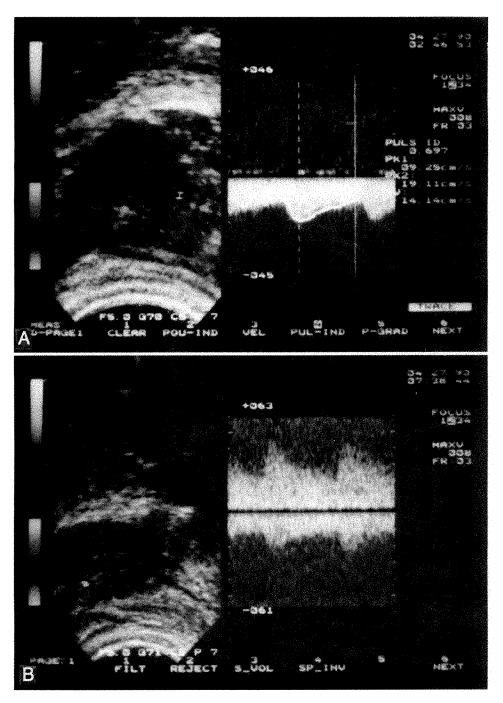


Fig. 1. A, A preovulatory follicle (at the time of the plasma luteinizing hormone peak) showing blood thaw velocity waveforms from the inner wall. B, Postrupture showing increased velocity and fuzzy waveforms. (Photograph courtesy of Dr. T. H. Bourne.)

A structure resembling the detached cumulus and oocyte is often seen before the follicle has started to rupture. More recently, transvaginal ultrasonography with color flow mapping is being used to study changes in intrafollicular morphology and blood flow during the periovulatory period. 9, 10 Blood flow velocity waveforms are observed clearly at the time of the rise in luteinizing hormone, and blood vessels are visible in the inner layer of the follicle (the granulosa layer) at the time of the luteinizing hormone peak. Concur-

rently, the wall of the follicle becomes less clear, crenated, thicker, and more echogenic. The peak systolic blood velocity frequency envelope is irregular (fuzzy) at this time (possibly indicating the presence of broken blood vessels), but becomes smoother as the process of ovulation develops. Accordingly, the visualization of blood vessels within the inner layer of the follicle and the presence of a detached oocyte and cumulus are most indicative of impending ovulation (Fig. 1), and the presence of fuzzy waveforms with high peak blood

velocity and a reduction in follicular size are consistent with the occurrence of the event.

Performance of current markers

The four main requirements of current practical markers are as follows: (1) to ascertain whether ovulation is likely to have occurred; (2) to identify the day on which the oocyte was released, for the retrospective dating of events during the ovarian cycle; (3) the immediate prediction of ovulation, to aid the treatment of subfertile couples; and (4) to identify the probable limits of the fertile period, which may be defined as the time from the fourth day before ovulation to the second day after the ovum has been released. Achievement of the last aim would be of value to couples who wish to practice family planning by periodic abstinence or the restricted use of barrier methods.

The following criteria should be considered before the performance of current markers can be assessed: (1) the type of test (including the aspect of the reproductive process being monitored); (2) the purpose of the test (i.e., the avoidance or achievement of pregnancy); (3) the age and menstrual status of the user (e.g., postpuberty, postpartum, or perimenopause); (4) the endpoints for evaluation (comparison with other tests, or pregnancy rates); and (5) the number of method or user failures.

Prospective, randomized trials are required to evaluate the clinical usefulness of each test for a specific application. The retrospective analysis of data suggests that the failure rates of methods could be low when used by motivated women of reproductive age (with regular menstrual cycles), who wish to avoid a pregnancy. Information is being collected on the potential usefulness of all tests in fully lactating women and in others approaching the menopause. At these times there are undoubtedly many false-positive and falsenegative signals of impending potential fertility. Similarly, there will be false-negative signals when the data are used by subfertile women hoping to achieve a pregnancy.17, 18 Possible signs and reasons for anovulation are being investigated, with the aim of incorporating other indices into the diagnostic procedures. A combination of tests will undoubtedly provide the best estimate of impending ovulation and potential fertility.

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Idiometric assay, the third way: A noncompetitive immunoassay for small molecules

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A novel, noncompetitive immunoassay applicable to the measurement of small molecules including ovarian steroids is described. Using monoclonal antibodies with the ability to recognize both β -typic and α -typic binding sites, a new simplified, sensitive, and specific immunoassay system has been developed. The initial work of this development is presented, along with preliminary results for a novel immunoassay for estradiol. (AM J OBSTET GYNECOL 1991;165:1997-2000.)

Key words: Ovarian steroids, noncompetitive immunoassay, monoclonal antibodies, estradiol

The pioneers of competitive radioimmunoassay were Yalow and Berson, who introduced a method for the measurement of insulin in 1960. Concurrently, Ekins introduced the terms "limited reagent method" and "saturation analysis" to indicate that the concentration of the binding protein was insufficient to bind all the analyte. More recently, however, he has discussed some of the limitations of competitive immunoassay, which include: (1) limited sensitivity and working range, (2) slow-reaction kinetics, (3) increased imprecision, and (4) the development of a negative endpoint (i.e., an inverse relationship between signal and analyte concentration).³

In 1968 Miles and Hales⁴ introduced a noncompetitive immunoassay that they termed immunoradiometric assay, which used isotopically labeled specific antibodies. Based on this principle, two-site, noncompetitive immunometric assays have largely replaced the earlier competitive methods for the measurement of large multiepitope analytes (e.g., protein hormones) in biologic fluids. The advantages of these excess reagent assays are greater sensitivity, precision, and working range of analyte.⁵ The major disadvantage, however, is their unsuitability for the measurement of small molecules.

Ekins⁶ has suggested that the fundamental difference between competitive and noncompetitive procedures is based solely on the detection of antibody occupancy. Accordingly, we have devised a way to detect antibody occupancy (i.e., a noncompetitive method) using two

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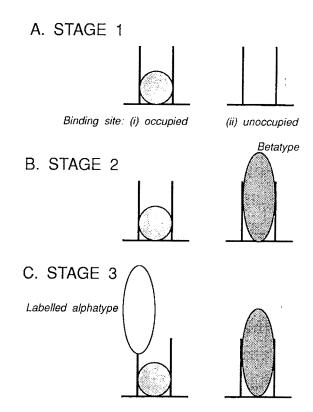


Fig. 1. Idiometric assay. Stage 1: Analyte capture to excess immobilized antianalyte immunoglobulin G, creating occupied and unoccupied sites; stage 2: β -type addition blocks unoccupied sites; stage 3: Addition of europium-labeled α -type detects occupied sites.

antiidiotypes with different epitope specificity. This procedure is outlined in Fig. 1.

The term "idiotype" was originally proposed by Oudin and Michel⁷ to designate antigenic determinants unique to a small set of antibody molecules. More recently, Jerne et al.⁸ have classified antiidiotypic reagents according to the location of the idiotype recognized. Two main types of antiidiotype have been distinguished

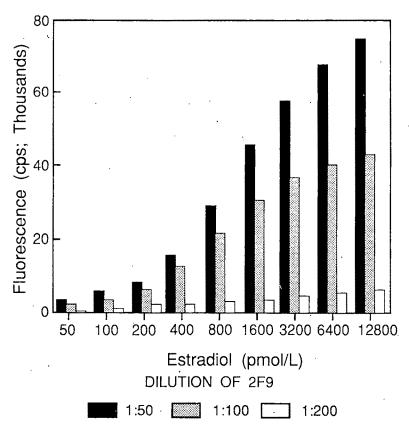


Fig. 2. Calibration curves at various dilutions of capture antibody (clone $2F_9$). Stage 1: 20 μ l of standard or sample in duplicate to the antiestradiol immunoglobulin G (clone $2F_9$)-coated microtiter wells containing 100 μ l assay buffer. Incubate and wash. Stage 2: 100 μ l β -type (clone $14H_{10}$) blocks unoccupied sites. Incubate. Stage 3: 100 μ l europium-labeled alpha-type (clone $1D_7$) addition. Incubate, wash, and fluorescence detection of occupied sites.

and these have been termed Ab2 α (α -type) and Ab2 β (β -type). By definition, β -types compete with the analyte for an epitope at the binding site (paratope). In contrast, the α -type recognizes a framework epitope and is not sensitive to the presence of the analyte at the binding site. In particular, the subset of α -types selected for the development of idiometric assay will not bind to the β -type/primary antibody complex because of steric hindrance resulting from epitope proximity.

To generate the antiidiotypic reagents, we immunized 2-month old female CD₂ mice with purified antiestradiol immunoglobulin G (clone 2F₉) in Freund's adjuvant according to a procedure previously described.⁹ After 6 months of immunization, the mouse showing the highest serum titer of antibodies recognizing europium-labeled antiestradiol immunoglobulin G was killed, and the immune spleen cells were fused in the presence of polyethylene glycol by the hybridoma technique of Kohler and Milstein.¹⁰

The initial screening procedure involved the binding of suspected immunoglobulin G from the culture supernatants of growing hybridomas to antimouse immunoglobulin G immobilized to the walls of polystyrene microtiter wells. After blocking the excess antimouse sites with nonspecific mouse immunoglobulin G, europium-labeled antiestradiol was added. Immobilized immunoglobulin G that bound the labeled reagent was indicative of a positive clone.

The second screening strategy involved the immobilization of the immunoglobulin G from the positive hybridomas. After blocking, we added europium-labeled antiestradiol that had been previously incubated in the presence and absence of an excess of estradiol (1 μ g/ml). Immobilized immunoglobulin Gs that failed to bind the europium-labeled antiestradiol in the presence of the analyte were classified as β -type antibodies.

The principle of the third screening assay is based on two-site epitope analysis similar to a procedure described elsewhere. The method involved the binding of the immunoglobulin G from the hybridomas that gave positive results in the second screening assay to the antimouse immunoglobulin G plates. After blocking, we added europium-labeled antiestradiol that had previously incubated in the presence and absence of

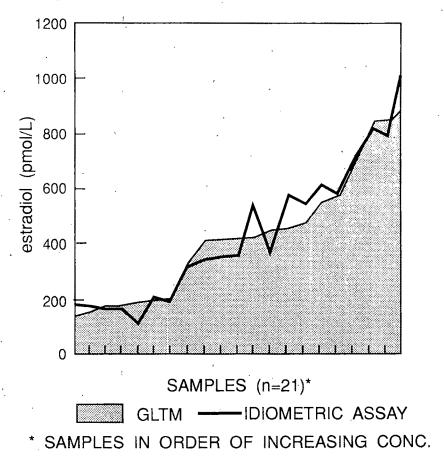


Fig. 3. Measurement of estradiol in serum. GLTM, Group laboratory trimmed means.

culture supernatants from hybridomas secreting strong β -types. Immobilized immunoglobulin G that failed to bind the europium-labeled antiestradiol in the presence of β -type was classified as an α -type.

Thirty-seven hybridomas secreting antibodies against antiestradiol (2F9) were obtained from the fusion experiment. Of these, eight demonstrated strong β -typic activity, three demonstrated weak β -typic activity, and four demonstrated α -typic activity. Appropriate clones of each class were selected and propagated as ascites in mice. The immunoglobulin Gs were purified, and the α -type was labeled with europium. 12

Using these reagents, we have successfully developed a noncompetitive, excess reagent assay system for the measurement of small molecules. This is illustrated for estradiol by the idiometric calibration curves shown in Fig. 2. In addition, we have measured the concentrations of estradiol in 21 quality control samples by idiometric assay and have compared the values with the group laboratory trimmed means. The results are shown in Fig. 3.

Currently, we are producing reagents for simple idiometric assays for the measurement of serum and salivary progesterone and urinary pregnanediol- 3α -gluc-

uronide and estrone-3-glucuronide. Furthermore, we are investigating the suitability of this approach for the measurement of peptides (e.g., buserilin) and proteins (e.g., human growth hormone).

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Home tests to monitor fertility

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Industry has been intimately involved in the development of products used in the monitoring of fertility at home. The perspective of industry and the development of such tests including their rationale are presented. The methods taken by industry to utilize current technology in the development of reliable, rapid, one-step tests for urinary human chorionic gonadotropin and luteinizing hormone and the subsequent expansion into other areas are covered. (AM J OBSTET GYNECOL 1991;165:2000-2.)

Key words: Fertility monitoring, home tests, monoclonal antibodies, enzyme-linked immunoassay

The concept of home/bedside testing is by no means new, and several references to this can be found dating back to the last century and even earlier. However, the gap between concept and reality can only be bridged by the advent of technology that is capable of being adapted to meet the needs of the consumer. The area of home diagnostics is currently limited to a small range of tests and is dominated today by two product categories: blood glucose monitoring and home pregnancy testing. Providing the technology that allows these tests to be routinely performed at home is a relatively recent phenomenon. The past 5 years have seen these products move even closer to the consumer's ideal as a result of a recent technologic advance.

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The evolution of home tests for fertility monitoring

Pregnancy tests were the first immunoassays to enter the over-the-counter market in the mid 1970s. In the early 1980s, most of the competing products were still based on red cell or latex agglutination. The tests were not reliable for most consumers, because they were difficult to use and interpret and were basically repackaged laboratory tests. The user was presented with a miniature chemistry set containing test tubes, reagents, droppers, and urine collectors. In 1984 market research showed that people were looking for a new test that was:

- Rapid (less than 30 minutes)
- Sensitive (results as early as the first day of the missed period)
- Easy to use
- Easy to read
- Hygienic (avoided the need to collect and dispense a sample)

The development of monoclonal antibodies in 1975 by Kohler and Milstein' and its subsequent adoption by commercial companies allowed for the first time the repeatable production of specific, high-affinity antibodies in gram amounts. This advance allowed the use of high levels of antibody and led to considerable enhancements in speed and sensitivity in diagnostic tests. The coupling together of monoclonal antibodies with dipstick enzyme immunoassay technology gave further improvements in ease of use and ease of reading the result. The first of these color end point tests was the Monoclonal Antibodies Inc. Pregnastick and saw the time to complete the test reduced to 30 minutes. A further evolution of this base technology was seen by the emergence of Clearblue, which also included a unique and patented sampling device that addressed for the first time the collection of a sample (May 1988).2 All solutions were dispensed ready to use in the base unit, removing the requirement for droppers and accurate dispensing of liquids. A measure of how far this moved toward answering the needs of the consumer was seen from the success of this product in the market.

The trend in technology development in the mid 1980s was, however, still toward faster and easier testing procedures. The quest for speed led many companies to investigate membranes as alternative solid phases to coated tubes or dipsticks. Indeed, some membranebased enzyme immunoassay products started to appear in the processional market in 1986 from Abbott and Hybritech. The Hybritech Icon reduced the time to complete a test to 5 minutes. The speed enhancement was largely achieved through the presence of high levels of antibody per unit surface area of membrane and a high surface area to volume ratio within the membrane. This leads to very short diffusional distances and pseudo first-order reactions.3 The tests were still multistep and used liquid reagents that had to be added with droppers. In addition, no attention was paid to sample addition and hence were unable to meet the criteria for an ideal test proposed by consumers.

Another technology that appeared to offer the potential for widespread application was enzyme immunochromatography.4 This technique, however, appears to have limitations on sensitivity and still required liquid reagents.

Moreover, while some of these technologies moved a little closer, in 1986 there was still a gap between these concepts and consumers' ideal product. The target defined by consumers at this stage was a test that was rapid: less than 5 minutes, required no manipulation by the user, maintained the sample collection and hygienic handling of Clearblue, and provided a clear, easy to read result. To achieve this, it was necessary to replace the enzyme labels with direct labels and to make the addition of sample bring together the reactants. The first pregnancy test to reduce the manipulations, including sample collection, to one step was Clearblue One Step, which was introduced in June 1988.

The product is genuinely a one-step pregnancy test-all the user has to do is to hold the absorbant sampler in the first morning urine stream, replace the cap on the device, and wait for 3 minutes.

Clearblue One Step utilizes monoclonal antibodies in a novel, rapid assay technology and was developed and patented by Unipath. It is the only truly one-step pregnancy test and also includes a unique, built-in control that shows when the test is complete (removing the need for timing) and that the test has been carried out correctly.

The essential features of the test are an absorbent wick that is held in contact with a porous membrane material. The membrane has three separate zones of antibody in it. These zones of antibody are deposited in such a way that the first is capable of being mobilized by the sample and the other two are immobilized. When the sampler wick is saturated with urine, this passes along the membrane material until it reaches the mobile zone of reagent. The mobile reagent consists of colored latex particles sensitized with monoclonal antibody to the α-subunit of human chorionic gonadotropin (hCG). The urine picks up the colored latex and carries it to the second zone located directly below the large window. The second zone is composed of monoclonal antibody to the \beta-subunit of the hCG immobilized in the membrane. If the urine contains hCG, the hCG will react with the anti-α hCG on the latex and will allow this to be trapped by the anti-β hCG zone, causing the formation of a blue line in the large window.

The latex and urine continue to move along the strip by chromatography and come into contact with the third zone (below the small window). The third zone consists of an antimouse immunoglobulin antibody that is immobilized in the membrane. Excess or unreacted latex passing the first zone will be trapped, leading to the appearance of a blue line in the small window every time whether hCG is present or not.

Recent advances

The technology has been extended to produce rapid one-step tests for hCG, streptococcus A, and Chlamydia under the tradename Clearview. The Chlamydia test is particularly worth mentioning, because it allows relatively unskilled users to detect Chlamydia in under 30 minutes compared with the traditional culture detection methods that take 3 to 5 days. The test provides

sensitive and reliable detection of *Chlamydia* in endocervical swabs.^{5, 6}

All these tests are qualitative yes/no tests in which detection of the presence or absence of the analyte is sufficient. The extension of the technology into semi-quantitative testing is illustrated by Clearplan One Step, which allows the user to determine when levels of luteinizing hormone (LH) have reached the preovulatory surge levels.

In the presence of basal levels of LH, the control line is much darker than the test line. As the LH level increases, the intensity of the test line increases, so that when the preovulatory surge occurs (i.e., levels of 30 mIU/ml of LH or greater), the test line is of similar intensity or darker than the control line. When used by the women at home the surge can easily be detected.

The use of simple reflectance reading instrumentation provides the potential for quantitative measurements of analyses. We anticipate that many of the advances that have been made during the development of other home test kits can be expediently applied to kits for monitoring fertility.

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A prototype for ovulation detection: Pros and cons

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A noninstrumented enzyme immunoassay for urinary estrone conjugates was adapted from an instrumented microtiter plate enzyme immunoassay assay. The end point of the assay was a color change from green to clear, which was visible to the unaided eye. The visible color change was adjusted to allow 80 ng/ml estrone conjugates (on the basis of a sample size of $6.5~\mu l$ urine) to be distinguished from an infinite dilution without instrumentation. The evaluation of human urine collected from ovulatory ovarian cycles demonstrated that early follicular phase concentrations ($35.9~\pm~6.8$ to $79.4~\pm~14.7$ ng/ml, n=10) produced a dark-green color, whereas late follicular phase concentrations ($162.9~\pm~20.1$ ng/ml, n=10) produced no color. Daily urine samples throughout 10 ovulatory ovarian cycles produced parallel profiles when compared to measurements of estradiol in paired blood samples. Complete analysis of the data indicated that ovarian follicular dynamics can be accurately monitored through the noninstrumented analysis of daily estrone conjugates in urine samples. (AM J OBSTET GYNECOL 1991;165:2003-7.)

The events associated with ovarian follicular growth and development traditionally have been characterized by the measurement of serum or plasma estradiol. More recently, radioimmunoassay (RIA) measurements of urinary metabolites have been shown to be practical replacements for measurements of blood concentrations of estradiol in human¹⁻³ and nonhuman primates. Urinary measurements of excreted hormone concentrations have been shown to provide an accurate appraisal of estrogen dynamics during both the normal and the abnormal human menstrual cycle as well as during the periimplantation period in human beings^{3, 5} and macaque monkeys.

At the present time, efforts are being made to provide nonradiometric assay substitutes for RIA methods. Enzyme immunoassays (EIAs) have been developed for urinary steroid metabolites. Urinary analysis of ovarian function has advantages over serum analysis because subjects can collect their own samples for lengthy studies and store them by freezing without preservatives for analysis at a central site. Enzyme assay formats have advantages over RIAs in that they eliminate toxic and regulated substances, reduce monetary and labor costs of analysis, and enhance the ability to expand study designs to include the monitoring of large numbers of subjects over relatively large geographic areas. Additionally, enzyme immunoassay formats provide

the basis for the development of noninstrumented assays that can be used under nonlaboratory conditions. Such assays would allow subjects to collect and analyze their own daily urine samples and, in certain situations, detect the fertile period or anticipate ovulation.

Commercial tests are currently available for the primary metabolites of progesterone, but there are few, if any, tests for impending ovulation. Most tests, like those signaling the luteinizing hormone (LH) surge, or change in the estrogen/progesterone ratio, allow detection or confirmation of ovulation only after the fertile period has begun. Such indicators can be used for fertility enhancement, but they have little value as an aid to prospectively avoid conception.

The best indicator of the fertile period is the initiation of the preovulatory estrogen rise. This event coincides with the selection of the ovarian follicle, which will ovulate 3 to 6 days later. If the initial rise in serum estradiol is predictive of ovulation by 5 days, then it would follow that urinary metabolites also can be used for the same prediction—assuming a minimal lag time between changes in serum estradiol and urinary metabolites. We have demonstrated a preovulatory rise in both serum and urinary estrogen, but have not correlated the events in urine to those in serum using a noninstrumented assay format that could be used to document follicular development and ovulation outside of the laboratory.

The purpose of this study was to evaluate the adaptation of an instrumented EIA for estrone conjugates (EIC) to a noninstrumented EIA (NEIA). Changes in urinary EIC concentrations measured by NEIA were compared to changes in serum estradiol concentrations in paired daily blood and urine samples throughout ovulatory menstrual cycles. Comparisons were made

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between estrogen concentrations in daily serum assessed by RIA and urine samples assessed by both instrumented and noninstrumented EIAs to determine the timing before ovulation of the first estrogen rise in each.

Material and methods

Subjects, samples, and previous analysis. The serum and urine samples used in this study were a subset of those described in a previous report.8 Subjects and collection of samples are described in detail in the previous report. Briefly, women between the ages of 23 and 40 years with normal menstrual cycles were recruited for the study. Daily early morning urine samples and midmorning blood samples were collected throughout one complete menstrual cycle. Samples were frozen and stored until evaluation. After evaluation, remaining urine samples were refrozen and stored for approximately 1 year. Urine samples for complete menstrual cycles of six subjects were then reanalyzed by the NEIA reported here. These ovarian cycles were previously shown to be ovulatory by the assessment of serum LH, follicle-stimulating hormone (FSH), estradiol (E2), and progesterone (Po) using commercial kits purchased from Diagnostic Products Inc. (Los Angeles, Calif.).8 In addition, cycles were analyzed for urinary ElC and progesterone (PdG) by validated laboratory methods described by Munro et al.8 Creatinine was measured by the method of Taussky (1969) to adjust for the differences in urine concentration. The day of the midcycle LH peak was used as a reference for the day of ovulation and a sustained rise of serum progesterone for 12 to 15 days was observed in all menstrual cycles studied.

Estrone conjugate noninstrumented enzyme immunoassay. The ElC NEIA is similar in format to the EIC EIA described by Czekala et al.6 and uses most of the materials reported by Munro et al.8 Three major changes were introduced to the microtiter plate EIC EIA format. High-binding star tubes (Nunc Maxisorp star tubes, Nunc Inc., Naperville, Ill.) were substituted for the microtiter plate as a solid matrix for the assay to maximize the color change during the midfollicular phase when the estrogen production of the dominant follicle signaled the beginning of the fertile period. Second, the estrone conjugate enzyme label was altered to maximize the dose response dynamic by eliminating the glucuronide moiety. This steepened the slope and provided an "all-or-none" or "binary" signal to be generated over a small change in concentration of standard or unknown. The 50% binding point of the standard curve of the ElC NEIA with estrone horseradish peroxidase as the enzyme competitor was 400 pg/tube (or

80 ng/ml on the basis of a sample size of 6.5 μ l urine) of estrone-3-glucuronide. Third, small Whatman No. 1 filter paper pads (7.5 mm diameter) were used to measure and transfer urine samples. Pads were dried completely and stored at 4° C until assayed. Several pad samples of individual urines were analyzed to test the reliability of sample volume measurement as well as the shelf life of the sample dried onto the absorbent pad. Reliability was comparable to micropipettors (6.5 \pm 0.3 μ l, n=12, coefficient of variation 1.6%); samples could be stored up to several weeks with refrigeration before analysis without loss in reactivity: one cycle was analyzed by EIC NEIA on four different occasions by use of sample pads that had been stored for more than 6 months at room temperature, with no change in results.

The EIC NEIA was performed by coating star tubes with 0.5 ml of antibody R522.8 After a minimum incubation of 18 to 24 hours, the tubes were rinsed with EIA wash. A clean filter paper pad was soaked in a urine sample for 1 to 3 minutes, blotted dry, and placed into 0.5 ml of the conjugate solution added to the precoated star tube. After a 60-minute incubation the contents of the tube was discarded, the tube was rinsed with EIA wash, and 0.5 ml of substrate was added. The visual color change was evaluated quantitatively by measuring the optical density of a 0.1 ml aliquot transferred to a microtiter plate and read on a Dynatech MR600 spectrophotometer (405 nm).

Statistics. Composite cycles were derived from mean daily hormone values of the individual subjects and were aligned according to the serum preovulatory LH/FSH peak. CUSUM analysis, as described by Royston⁹ was used to describe the pattern of urinary estrogen concentrations before ovulation. The "first rise" of serum E₂ or urinary EIC was defined as that day before the LH surge on which the mean for all cycles was greater than a reference value. The reference value was defined as "baseline plus 0.3." The "baseline," in turn, was defined as the mean of the first 6 days of each individual cycle expressed as a natural log. A significant rise was defined as that day before the LH/FSH peak on which the value exceeded the "decision interval" (0.6).

Results

When compared with serum E_2 measurements, the urinary ElC EIA values lagged behind by 0.7 day, indicating that less than 1 day was lost when the urinary dynamic of the ElC was measured in place of serum E_2 . The composite urinary ElC profile determined by EIA and the serum E_2 (concentration measured in picomoles per liter) profile determined by RIA for the same 10 cycles are shown in Fig. 1. Although composite

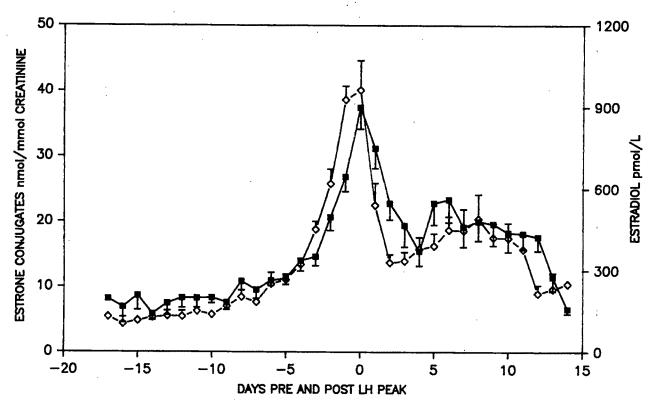


Fig. 1. Daily mean serum estradiol and urinary EIC concentrations in 10 ovulatory menstrual cycles. (From Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Clin Chem 1991;37:838-44, with permission from Clinical Chemistry.)

peak values for both EIC and E2 occurred on day 0, four of 10 cycles had E_2 peaks on day -1 with an ElC peak on day 0, and two had E2 peaks on day 0 and EIC peaks on day +1. As previously reported, serum E₂ values showed a postovulatory nadir on day +2, and ElC values in urine showed a postovulatory nadir on day +4.3 Both serum E2 and urine ElC concentrations increased during the luteal phase, remaining at about 50% of peak values until approximately day +10 before declining to early follicular phase levels. The mean urinary ElC profile was parallel to the mean serum E2 profile (r = 0.88, p < 0.001).

Estrone conjugate evaluations of human urine by EIA indicated that EIC concentrations in the early follicular phase ranged from 35.9 ± 6.8 to 70.4 ± 14.7 ng/ml, whereas late follicular phase concentrations were as high as 162 ± 20.1 ng/ml. For the ElC NEIA to be of diagnostic use, a significant color change had to occur at the periovulatory estrogen excursion (i.e., at 70.4 ± 14.7 ng/ml urine). The high-binding star tubes showed a distinct visual color change at approximately 70 to 80 ng/ml based on an absorbent pad sample of 6.5 µl urine. Under these conditions, early follicular phase concentrations exhibited dark-green coloration, whereas late follicular phase and ovulatory concentrations were indicated by no color. In general, the dark-green color progressively lightened over two or three samples 3 to 4 days before the LH/FSH peak and was nearly colorless in all cycles on the day of the ElC peak, as determined by instrumented EIA.

When six ovarian cycles evaluated by NEIA were assessed quantitatively by transferral of both the standard curve and unknowns from the star tubes to a microtiter plate for reading the results on a spectrophotometer, a composite profile was obtained that was identical to that produced by the original EIC EIA for 10 cycles. The individual profiles of serum E2 and urinary ElC as measured by NEIA—like those measured by instrumented EIC EIA—were parallel (r = 0.88, p < 0.01).

Statistical analysis by CUSUM for the six menstrual cycles evaluated for both E2 by RIA and ElC by EIA and ElC NEIA are presented in Table I. The "first rise" is defined in CUSUM analysis as the day before the LH surge that the CUSUM becomes positive—i.e., above the reference (baseline + 0.3). Baseline is calculated as the mean of the first 6 days of each cycle and was expressed as loge, and significance is determined as the

Table I. CUSUM results for serum E₂(pg/ml), urinary E1C as measured by instrumented enzyme immunoassay and indexed by creatinine (EIA/CR), and urinary E1C as measured by the noninstrumented format and indexed by creatinine (NEIA/CR)

•	*			•				
	1	2	3	4	5	6	Range	Mean
Serum E ₂								,
First rise	-3	-8	-8	-7	-5	-5	-3 to -8	-6
Significance	-2	-6	-6	-6	-4	3	-2 to -6	-4.5
Urine EIC EIA/CR			•					
First rise	-2	-8	-6	-7	-4	-6	-2 to -7	-5.5
Significance	- 1	-6	-5	-6	-2	3	-1 to -6	-3.8
Urine E1C NEIA								
First rise	0	-7	-6	-6	02	-4	0 to -7	-5
Significance	+2	-1	-6	-6	 1	-3	+2 to -6	-3.4

(From Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Clin Chem 1991;37:838-44, with permission from Clinical Chemistry.)

day before the LH surge that the CUSUM exceeds the decision interval (0.6). With these CUSUM criteria, daily serum E₂ concentrations accurately predicted the fertile period in five (86%) of the six cycles analyzed. Urinary ElC measured by microtiter plate EIA and indexed by creatinine gave similar results when the 1 day lag in excretion compared to serum E₂ secretion was considered, with a mean detection of 5.5 days, confirming that urinary ElC can be used in place of serum E₂ to predict the fertile period. Essentially the same results were obtained with the ElC NEIA as with the microtiter plate ElC EIA without indexing by creatinine.

Comment

The present study was conducted on the basis of historic data that indicate that the increase of serum E₂ concentrations accurately and consistently predict the occurrence of impending ovulation. The data presented here support that indication and demonstrate that, with early morning samples, changes in urinary estrogen excretion during the normal ovarian cycle can also be used to predict ovulation. In addition, urinary estrogen metabolites can be detected with an NEIA and that these measures also predict the ovulatory event. Although the numbers of observations are limited, a consistent rise of urinary ElC was detected as early as 6 days and as late as 2 days before the midcycle LH peak, which was comparable to the rises detected when E2 was measured in paired blood samples. In general, E₂ measurements provided an earlier and more consistent prediction of the LH surge, but the advantages of the ElC NEIA and the use of urine more than compensate for the small difference lost in predictiveness. The immediate use of this assay is to provide a selfevaluation of the major ovarian follicular events and predict ovulation several days in advance.

In five of the six cycles monitored by EIC NEIA, the preovulatory estrogen rise in urine was detected at approximately the time of the beginning of the fertile period, which is considered to be 5 days before ovulation or 4 days before the LH surge.³ It would appear therefore that this technique has the potential for prediction of ovulation by several days. This information could be used for either the timing of propitious insemination or for the anticipation of the fertile period so as to reduce the risk of pregnancy at this time.

When EIC values were combined with the measurement of urinary progesterone and luteinizing hormone metabolite measurements, the detection of the EIC rise during the follicular phase provided the ability to completely and comprehensively monitor all major ovarian events of the complete menstrual cycle. The ability to generate such information may in turn provide the basis for more detailed future studies of ovarian function in contexts in which biologic samples other than urine cannot be collected and stored for later evaluation. The EIC NEIA presented here is particularly useful when overall cycle lengths are variable or when even a general estimate of the day of ovulation cannot be made.

The inherent variations in urine concentration, which are compensated for in the laboratory by indexing each hormone value by the creatinine concentration, can, in the extreme case, contribute to false information. Whereas this is not a problem with the measurement of urinary luteinizing hormone or progesterone metabolites in which large increases of signal is exhibited over the baseline values, the relatively small baseline to peak ratio of the EIC profile is perturbed by variations in urine concentration that can be seen under normal conditions: false-positive and false-negative EIC NEIA results are to be expected when urine

osmolality is either high or low, respectively. A safeguard against such errors would be to regulate fluid intake and therefore normalize urine production during testing periods. Alternatively, urines could be tested with a simple device simultaneous to the NEIA to ensure that each urine concentration falls within a prescribed range. It seems likely that this aspect of estrogen monitoring will require the development of a noninstrumented test for urine osmolality in the near future.

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Use of the Home Ovarian Monitor in pregnancy avoidance

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The application of the Home Ovarian Hormone Monitor to the avoidance of pregnancy by periodic abstinence has been explored. No woman had difficulty with the daily urine testing, and their results consistently identified the distinctive hormone pattern of the ovulatory cycle and the day of ovulation and correlated closely with the mucus symptoms. The tests gave 4 days or more warning of ovulation in 99% of cycles and allowed intercourse to be resumed 1 to 3 days after ovulation in 88%, giving a mean period of abstinence of 7 days. No pregnancy occurred from intercourse during the late safe days defined by the Monitor, but some early-day pregnancies occurred through long sperm survivals of 6 to 8 days, mostly during the return of fertility after breastfeeding. Rules for the avoidance of pregnancy, with the minimum of testing on the basis of these results, are given. (AM J OBSTET GYNECOL 1991;165:2008-11.)

Key words: Urinary estrogen, progesterone metabolites, fertile period, home test, Ovarian Monitor

The Ovarian Monitor measures estrone glucuronide (EIG) and pregnanediol glucuronide (PdG) separately by homogeneous enzyme immunoassay with final readout in a specially designed meter. The Ovarian Monitor and its validation have already been described. Its application to the home monitoring of ovarian responses during gonadotropin therapy and the close agreement between the results of home and laboratory testing have been published recently.²

This article presents the results obtained during the past 3 years in the application of the Monitor by women at home for avoidance of pregnancy.

Methods

The rules for use of the Monitor for avoiding pregnancy are shown in Table I. The ElG baseline and the first ElG rise are determined during the first cycle tested by essentially the same procedure as for establishing the rise in basal body temperature. The rise is usually quite definite. When in doubt, however, the women would abstain, but for the present assessment, the next more definite day was taken. Furthermore, particularly in long cycles, several rises may occur before the ovulatory rise takes place. In such cases, the day of the rise was the one that proceeded directly to the preovulatory ElG peak and fall, immediately followed by the PdG rise that reached the "cutoff."

The PdG cutoff was established from an earlier data bank of 104 cycles that showed that no woman who reached 6.3 µmol/24 hr after an estrogen peak and fall accompanied by a pregnanediol rise ever showed evi-

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dence of ovulation later in the cycle. This observation has been confirmed in the present study and has led to the confidence that is now placed in the definition of the postovulatory infertile days (late safe days) by these criteria.

The three-quarter rule (see Table I under Breast-feeding) was introduced for breastfeeding women and perimenopausal women who have a deficient luteal phase and whose PdG values never reach cutoff.

Results

The results obtained in a 3-year study involving 37 women who used the Monitor for pregnancy avoidance over a total of 661 months or 55 women-years are shown in Table II and Figs. 1 and 2. Fig. 1 shows the correlation between the day of the first ElG rise, the day of first mucus production, the day of "peak" mucus symptom, the day of the preovulatory ElG peak, and the day of the PdG cutoff in 12 women over 264 menstrual cycles up to mid-1989. Notice the close correlation between the first day of the ElG rise and the first day of mucus production; the days almost coincided in every case—the close fit of "peak" mucus with the ElG peak that occurs on the day before ovulation and the cluster of the PdG cutoff days on days 2, 3, and 4 after the ElG peak. The correlations between estrogen values and mucus observations are almost the same as those reported for laboratory measurement.3.4 The close correlation between the hormone values and the mucus symptoms the women can now observe provides them with important reassurance that both are correct.

Fig. 2 shows the more recent results produced by 33 women for 305 cycles. These included early ovulatory cycles from 12 breastfeeding women who had already been monitored for a total of 92 months during lactational amenorrhea. The emphasis in Fig. 2 is on the period of fertility as defined by the ElG rise and the

Table I. Rules regulating use of the Ovarian Monitor and the results it provides for avoiding pregnancy through periodic abstinence

General rules 1. Low E1G ("baseline"), low PdG 2. Raised E1G, low PdG	· ·		* .	,		-	Infertile Fertile
3. Raised PdG Cycling women	ć	•			,	,	Infertile

Establish early E1G baseline before resuming intercourse; use basic infertile mucus pattern (BIP) as backup
 Abstain when E1G rises above baseline and/or BIP changes

- 3. EIG rises to a peak and then falls; watch for the fall; this marks the day of ovulation and is the most fertile day of the cycle
- 4. Change to PdG measurement; watch for the PdG rise to cutoff (7 mmol/24 hr); can resume intercourse immediately; no further measurement or observation is needed in this cycle

Breastfeeding

1. Measure E1G once a week until 6 mo and twice a week between 6 and 10 mo; all days of persistently low baseline E1G values are available for intercourse; observe BIP for additional information

2. If E1G values rise, test more frequently and abstain

- a. If they rise marginally and return to baseline, intercourse can be resumed after 3 days; be careful; watch for the next rise
- b. If they rise above T 180 then fall, test for ovulation with PdG; intercourse can be resumed when PdG reaches cutoff or thirty-four cutoff if cutoff has not been reached on the third day after the E1G fall; from then on, monitor as for ovulating women

Table II. Results from a 3-year study of the use of the Ovarian Monitor as an aid in defining the fertile period for avoidance of pregnancy through periodic abstinence

No. of women	37	1
No. of menstrual cycles of use	569	1
No. of months of amenorrhea in breastfeeding users (12 subjects)	92	i i
Total No. of months of use	661	
No. of unplanned pregnancies	4*	1
Pear index for use-effectiveness	7.3 pregnanc	cies per 100 women-years

^{*}One of the pregnancies resulted through inappropriate use of the three-quarters rule.

PdG cutoff. In this series, most of the assays were performed on late afternoon 3- to 4-hour specimens of urine so that the decision to have intercourse was based on immediately preceding hormone values. In this case, the latest act of evening intercourse before the period of fertility would occur on the day before the ElG rise, and the first act of intercourse after ovulation would occur on the same evening the PdG cutoff was reached. Ovulation is assumed to occur on the day of the ElG fall after the preovulatory peak. Too long a warning provided by the ElG rise would result in unnecessary abstinence.

It must also be appreciated that ovulation is a 15-minute event at the longest and that daily monitoring cannot be more precise than 12 hours in timing this event. Fig. 2 shows that the ElG rise gave a minimum of 3 days' warning of ovulation and 4 to 8 days' warning in the majority. This would seem to be an excellent result when most workers consider that 3 days' warning would be adequate. In 28 cycles, no early safe days were provided. In the great majority of cycles, the PdG cutoff occurred on days 1, 2, or 3 after ovulation, but in six it occurred on the presumed day of ovulation without conception. One woman contributed three of these cycles and was recording intercourse on that day.

There are several explanations for these findings.

The most likely is that day 0, as defined by the ElG drop, was in error by a day because of the daily sampling and that the PdG cutoff provided the more accurate information on ovulation. Furthermore, the high PdG values reflect high progesterone values that themselves may be contraceptive, like the progestogen in combination oral contraceptive.

The important points to be noted are (1) the PdG cutoff and protection from pregnancy occur frequently on the day after ovulation, (2) the results emphasize the reliability of the test that distinguishes the day of maximum fertility in a cycle from the next day of total infertility, and (3) the median length of abstinence required by the Monitor was 7 days.

Comment

This series of 661 months of observation in pregnancy avoidance resulted in four pregnancies, representing a pregnancy rate of 8 per 100 women-years (see Table II). The exact sequence of events that occurred in the conceptual cycles were clearly revealed by the Monitor results, and they provided much of the important information summarized in the conclusions.

The women in the study were not selected by socioeconomic status, none was deemed unsuitable for training, and none had difficulty in performing the test.

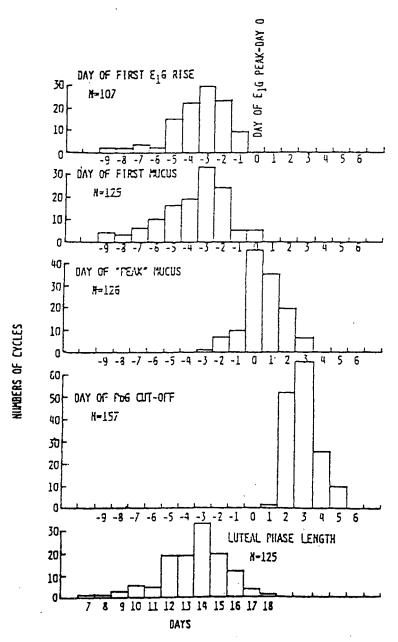


Fig. 1. Correlation between the day of the first ElG rise, the day of onset of fertile mucus symptoms, the day of the "peak" mucus symptom, and the day of the PdG cutoff in 12 women over 264 menstrual cycles.

The majority were more than 30 years of age with three or more children and had therefore completed their families and wanted a secure method to avoid pregnancy. Their views on the test and its use were sought by means of a questionnaire. Most considered the test a nuisance and they would like the work load to be reduced, although this was not a sufficient reason for stopping because of the sense of security it provided.

Most women combined the hormone values with the mucus symptoms to define days of potential fertility. Sixty-two percent were having sexual intercourse during the early safe days. All were using the late safe days; 21 used late safe days immediately after the PdG cutoff was reached and 13 added several days according to

the mucus rules for additional safety. Some women had difficulty remembering the times of urination, and some measurements were lost because it was too late to collect another specimen.

Although most subjects preferred doing the tests during evenings, social activity could create difficulties, particularly on crucial days when the measurement could not be left until the next day. Most of these objections have been accommodated by teaching the women to organize the testing to fit in better with their daily activities.

Home and lay-center usage of the Monitor throughout Australia added to the present series now amounts to 300 women-years of experience. On the basis of feed-

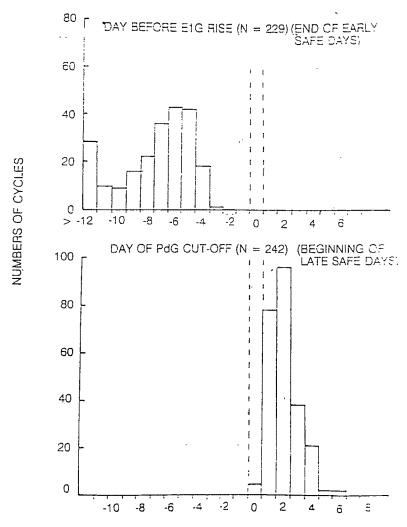


Fig. 2. The confines of the fertile period as defined by the urinary ElG rise and urinary PdG cutoff. Day 0 is the day of the ElG peak value. (Illustration provided by Georgetown University.)

back from this experience, we believe the Home Ovarian Monitor is an important advance in fertility. For couples who did not report infertility, the conception rate for intercourse within the fertile period, as defined by the Monitor, is much higher than the generally accepted figure of 25% per cycle. It is nearer to 70%.

In addition, 5-day sperm survivals to conception are not uncommon; 6-day survival is less common. The longest interval encountered was 8 days (1 in 4000 cycles). The last day of the ElG baseline and BIP is still the problem day; most unplanned pregnancies occurred from intercourse on this day. Women are highly fertile during the second and third ovulatory cycles after 10 months or more of breastfeeding. Appreciation of these findings has made unplanned pregnancies in our user groups a rarity. The Monitor is now available for others to test these conclusions and determine the effectiveness rates in independent studies.

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Discussion and recommendations

This session dealt with a variety of topics ranging from new advances in ultrasonography to detect subtle intraovarian changes occurring around the time of ovulation to the presentation of a fully self-sufficient home monitor that allows women to ascertain, in the privacy of their home, their relative fertility status. Described methodologic advances will ultimately allow further simplification of such home tests.

The papers presented demonstrate the considerable effort that has been made in the field of home testing for fertility. Despite this, it is also clear that many of the advances made have yet to be translated into useful simplified tests. Clinical trials are still desperately needed to determine the overall use of such tests for the regularly cycling woman as well as for women in special reproductive statuses (e.g., breastfeeding and perimenopausal). During the next 5 years, the Institute for International Studies in Natural Family Planning plans to fund further studies into the development of simplified tests as well as conduct clinical trials to determine their use. At the same time, we hope to encourage industry to increase their efforts in this same area. Many of the stumbling blocks that need to be overcome are related to the reluctance of industry to

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pursue this area with vigor. This reluctance is obviously because of marketing and patent issues as well as the possible product liability implications of such tests being in the hands of individual couples. It is clear that public education in the realm of fertility awareness will have to go hand-in-hand with the development of tests that allow the self-monitoring of fertility. Close interaction between couples and their physicians will be necessary.

The papers presented reflect advances in our understanding of the process of ovulation, new reagent development for ultimate simplification of the measurements of small molecular weight molecules, results from current available tests, and industry-sponsored advances in assay configuration and simplification. It is becoming apparent that we are approaching the time when simple tests to monitor urinary excretion of estrogen and progesterone metabolites are feasible. In addition, the use of such tests appears very promising. Although considerable work still lies ahead, it is anticipated that new clinical trials of recently developed tests will be underway in the next few years. The Institute for International Studies in Natural Family Planning plans to be at the forefront of these developments and to work to make such a home test, in cooperation with industry, a reality.

SESSION III. THE INTERFACE OF BREASTFEEDING, NATURAL FAMILY PLANNING, AND THE LACTATIONAL AMENORRHEA METHOD

Miriam H. Labbok, Chair Alfredo Pérez, Co-Chair Peter Howie, Rapporteur

Overview and summary: The interface of breastfeeding, natural family planning, and the lactational amenorrhea method

Miriam H. Labbok, MD, MPH, and Peter W. Howie, MDb

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This session explores the use of natural family planning during breastfeeding and the operationalization of the research findings related to the breastfeeding-natural family planning interface into training programs. Worldwide, the vast majority of women breastfeed their infants; in many countries breastfeeding continues for several months. Studies show that women learning natural family planning during lactation, particularly those whose menses have returned, have an increased risk of unplanned pregnancy. Altered hormonal levels may make interpretation of the signs of fertility return (mucus, and so forth) difficult during this time. The lactational amenorrhea method may be a useful adjunct to natural family planning training. (AM J OBSTET GYNECOL 1991;165:2013-4.)

Key words: Natural family planning, breastfeeding, lactational amenorrhea method

This session explores two issues. The first is the use of natural family planning (NFP), especially the ovulation method, during breastfeeding. Worldwide, nearly 100% of women will breastfeed their infants for some period of time. Therefore we must be able to introduce NFP during lactation and do so in a manner that is efficacious. Previous studies show that women learning the method during lactation are at risk of increased unplanned pregnancy rates. The period of lactational menses (i.e., the time when lactation continues but after menses have returned) is apparently the time when unplanned pregnancy is most likely to occur.

The second issue that will be discussed is whether better understanding of lactational amenorrhea could be part of the solution to the problem noted above and incorporated into NFP teaching. We know that breast-feeding patterns can have a profound effect on fertility and that the lactational amenorrhea method (LAM) is an efficacious and natural method for postpartum lactating women. Many commonalities exist between NFP and the LAM. First, both are natural methods that re-

quire no additional medication or insertion of a medical device. Also, both are highly user dependent and require instruction and counseling of the acceptor. Neither method is logistics dependent, and once taught and fully understood, these methods belong to the client for life. Thus these methods are extremely desirable in situations in which other family planning methods are not readily available or are not acceptable.

During this session, a clear consensus emerged among all the speakers and the discussants that LAM should be incorporated into NFP programs. The practical problem is to provide support and counsel to those mothers who wished to use lactational amenor-rhea as an important contribution to effective birth spacing.

Another issue identified during this session was the difficulty of interpreting classic signs of NFP during breastfeeding. The difficulty of interpreting NFP signs was a particular problem after the return of menstruation during breastfeeding but before the resumption of regular cycles, which is generally the phase between the end of LAM and the resumption of regular cycles.

It is also clear that in some communities, lactational amenorrhea will be an important method of fertility control for much longer than the 6 months recommended in the Bellagio guidelines. More work is required to identify those individuals or communities and the breastfeeding practices that are associated with the

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longer lactational amenorrhea. These issues only served to reinforce the consensus among all speakers that the LAM must be incorporated more closely into current NFP programs throughout the world.

It is important to recognize that breastfeeding, especially exclusive breastfeeding, has a profound effect on fertility and on the estrogenic changes that create the signs and symptoms used in various NFP methods. Lactation after menses has returned is not nearly as

protective against unplanned pregnancy. Better understanding of the fertility suppressive effects of lactation and its incorporation into NFP teaching could be part of the solution to this problem.

The following articles and discussions explore these and related issues. It is hoped that the conclusions of this session will provide guidance for NFP policy makers and trainers in the introduction of NFP during breastfeeding.

Is the lactational amenorrhea method a part of natural family planning? Biology and policy

Barbara A. Gross, PhD

Westmead, Australia

The lactational amenorrhea method is a natural method of family planning for women who breastfeed their infants. The underlying physiology results in a natural suppression of ovulation, and the concomitant amenorrhea, induced by exclusive (or almost exclusive) breastfeeding. This in addition to the infant's age of 6 months or less and specific feeding pattern are the parameters used to identify the possible return of fertility. The lactational amenorrhea method provides at least 98% protection against pregnancy. Data from a recent multicenter study of breastfeeding support the use of the lactational amenorrhea method as a natural family planning method. The lactational amenorrhea method can be incorporated into natural family planning programs and teaching. (AM J OBSTET GYNECOL 1991;165:2014-9.)

Key words: Natural family planning, breastfeeding, lactational amenorrhea method, postpartum infertility

The natural child-spacing effect of breastfeeding has been long recognized to be associated with amenorrhea and the duration of breastfeeding, particularly full breastfeeding. Furthermore, in population studies only about 5% to 10% of women have been reported to become pregnant while in lactational amenorrhea. This effect received little attention among populations where breastfeeding was not a common practice. However, the incidence and duration of breastfeeding have been changing in both developed and developing countries, particularly since the early 1970s. In developed countries there has been a return to breastfeeding, and well-educated mothers in the higher socioeconomic groups are more likely to breastfeed and breastfeed longer than those from the lower socioeconomic or im-

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migrant groups.⁷ In some developing countries, particularly in the urban areas, the pattern is the opposite; the more highly educated are less likely to initiate breastfeeding or to breastfeed for only short periods. In rural areas breastfeeding is more prolonged, but there has been a suggestion of a decline in the initiation and duration of breastfeeding in many populations. Economic changes and recent programs for the promotion and support of breastfeeding are associated with abatement and reversal of the decline in many settings.

The duration of lactational amenorrhea in urban and rural populations also differs markedly from country to country, with a range varying from 2 to 3 months up to 2 to 3 years. In many of these populations breastfeeding has provided the major control of fertility; it delays the return of bleeding/menstruation and ovulation, but it does not permanently prevent pregnancy. The impact of a decline in the duration of breastfeeding and its concomitant effect on lactational amenorrhea, particularly in developing countries, has been high-

lighted by several writers.⁸ For example, in Senegal, where the average duration of breastfeeding and lactational amenorrhea is 19 and 15 months, respectively, a 50% reduction in the duration of breastfeeding would require an increase in contraceptive use from 11% to 35% to maintain current fertility rates.⁹

Natural family planning programs and lactational infertility

Breastfeeding has not been recognized for its important natural effect on fertility or as a natural family planning (NFP) method in most NFP programs in spite of the knowledge presented above. Many NFP programs recognize a 7- to 12-week period of natural infertility after childbirth, which is extended by breastfeeding. This is based on studies of breastfeeding women in which basal body temperature^{10, 11} and endometrial biopsy¹² were used as indicators of the probability of ovulation preceding the first postpartum bleed. In these studies most of the women breastfed for 3 to 6 months, with the period of full (or exclusive) breastfeeding even shorter, and solid and liquid supplements (cereal and juice) were introduced as early as 3 to 6 weeks after delivery.

Studies by Gross and Eastman¹³ and Brown et al.¹⁴ compared mucus symptoms with serum levels of estrogens and progesterone or their urinary metabolites. They found longer periods of infertility associated with longer durations of full breastfeeding. These findings were incorporated into teaching programs associated with the ovulation method and the symptothermal methods developed in Australia. These programs suggest that couples begin observing and recording their signs of fertility from 3 to 5 weeks after delivery. If a couple wants an almost zero risk of pregnancy, they are often counseled to abstain from the seventh or tenth postpartum week until they have evidence of ovulation by a sustained thermal shift of at least 4 days, thus resulting in a long period of abstinence during a period of relative infertility.

Another choice presented by some NFP programs is to wean the baby to facilitate the return of cyclicity and the application of NFP. In other instances couples using NFP methods based on cervical mucus observations are instructed that they can be guided by their observations to determine the days of infertility and possible fertility and to apply the rules for avoiding pregnancy. The mucus signs can be confusing for some, however, and do not always reflect the underlying ovarian activity; signs may suggest ovulation when it is not occurring or fail to indicate ovulation when it does occur. This can result in unnecessary and prolonged abstinence, although this may be acceptable to a couple with a strong desire to avoid another pregnancy. At the same time, pregnancy is possible, although not probable. The short

duration of lactational amenorrhea along with a suggested high incidence of first ovulatory bleeds, in spite of low pregnancy rate, were the major influences on NFP programs of the 1970s.

Essentially the western breastfeeding patterns of the 1970s forced on NFP teaching nonacceptance of lactational amenorrhea as a natural method of family planning. Teaching programs with this viewpoint have been extended to the developing countries, where there has been and still should be a reliance on lactational amenorrhea as an effective natural method of birth spacing but with some guidelines as suggested below.

Lactational amenorrhea

The mechanism of lactational amenorrhea is still not well understood. Prolactin levels are elevated during pregnancy and continue to be elevated above normal prepregnancy levels during lactational amenorrhea, but they gradually return to normal as ovarian function returns. Prolactin levels increase in response to suckling and may be a marker of the altered hypothalamic-pituitary axis or may have a direct action on fertility, perhaps at the level of the ovary. Ovulation is prevented or follicular development may be altered, resulting in deficient corpus luteum function, altered endometrium, and infertility. This inhibition of ovarian function can continue even after bleeding resumes, explaining in part the lower than expected pregnancy rate while breastfeeding continues.

More recent studies15-18 have shown that menses can be delayed on average for 7 to 9 months even in wellnourished western women. Table I summarizes Australian studies indicating the return of vaginal bleeding between 33 to 48 weeks (7½ to 11 months) after delivery, with individual women resuming ovulation as early as 12 weeks and others delaying up to 2 years. Twentyfive percent to 30% of the women resumed menstruation while fully breastfeeding, with varying frequency and duration of breastfeeding episodes. In other populations such as in Chile and Mexico, the duration of amenorrhea is shorter in spite of full breastfeeding of high frequency. In most of the recent studies, 25% to 30% of women have evidence of ovulation (as indicated by adequate levels of serum progesterone or urinary pregnanediol) before the first bleed, but many of these ovulations show evidence of inadequate luteal function.17 Furthermore, the incidence of ovulatory first bleeds occurring during full breastfeeding and in the first 6 months has been shown to be low, with a suggested pregnancy risk of less than 2%.19

The lactational amenorrhea method

An individual couple can rely on breastfeeding to provide natural protection against pregnancy for at least 6 months, provided the mother has no return of

Year	N	Weeks (mean ± SD)	Study population	Туре	Study sponsor
1976-78	89	41.3 ± 25.5 (5-120)*	Australia	Retrospective, cross-section	Ford/Rockefeller
1976-80	29	48.4 ± 25.8 (14-105)	A.C.T.	Prospective	Ford/Rockefeller
1986-88	25	32.8 ± 16.1 (12-66.4)	Sydney	Prospective	FHI
1987-89	16	43.3 ± 12.3 (21-66.5)	Sydney	Prospective	WHO
1989-90	119	38.2 ± 38.4 (<13->52)	Sydney	Retrospective, last breastfed child	WHO

Table I. Duration of lactational amenorrhea (Australian studies, Gross and Eastman¹⁸)

FHI, Family Health International; WHO, World Health Organization.

bleeding (ignoring any bleed for up to 56 postpartum days) and the baby is being fully, or almost fully, breastfed. These guidelines provide less than a 2% risk of unplanned pregnancy.

The above statement is now often referred to as the *Bellagio Consensus Statement*.^{19, 20} When put into practice, the statement defined the lactational amenorrhea method (LAM)—a natural method of family planning based on the lactational amenorrhea produced by the ovulation suppression effects of full breastfeeding during the first 6 months of life. This method can be illustrated by an algorithm, or decision tree, developed initially by Miriam Labbok²¹ (Fig. 1) and modified according to the results of the recent research that formed the basis of the Bellagio Consensus Statement.²²

The Bellagio Consensus Statement was issued from a meeting of 25 scientists in Bellagio, Italy, convened by Family Health International and the World Health Organization. The Consensus Statement's pregnancy rate of less than 2% was based on data from three prospective studies of pregnancies occurring during lactational amenorrhea and full breastfeeding in the first 6 postpartum months.19, 20 This figure was then supported by the calculation of the probability of pregnancy based on observed premenstrual ovulation rates during the first 6 months of lactation. The pregnancy rate was assumed to be 25% of the ovulation rate, although fecundity of ovulation during breastfeeding is thought to be much lower than 25%. With this high estimate for comparison, data from 10 small prospective studies (25 to 113 subjects) using hormonal evidence of returning ovulation gave estimated pregnancy rates up to 2.9%.20

The Bellagio Consensus Statement indicates that if these guidelines are adhered to (i.e., LAM is used), then 98% to 100% of couples will have warning of impending ovulation and can then make a decision on an alternative method of family planning, including NFP.¹⁹

Three-center breastfeeding/NFP study

Preliminary data from a three-center study of NFP and breastfeeding in the United Kingdom, Canada, and Australia are described in more detail in the another article in this issue (Kennedy et al.).

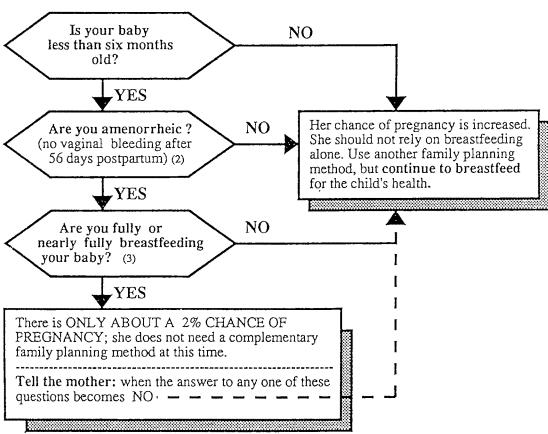
Table II illustrates the mean days of first bleed, first ovulation, and average number of days of protection provided by the Bellagio guidelines. The mean day of first bleed was very similar in Australia and Canada (229.6 \pm 112.9 and 221.04 \pm 88.5 days, respectively), and this was approximately 30 days longer than in the United Kingdom (198.9 \pm 114.5 days). The average number of days of infertility provided by LAM/Bellagio guidelines was 134, 132, and 112 days, respectively, for Australia, Canada, and the United Kingdom.

In all cases in Australia, the last day of protection provided by the Bellagio guidelines preceded the estimated first day of ovulation. In one case the first "bleed" was actually 6 days of spotting. If the spotting were discounted, one adequate and normal ovulation preceded both the Bellagio cutoff the first bleed in one woman who was fully breastfeeding. In addition, the first ovulation was not always an adequate ovulation as defined by pregnanediol levels (i.e., >9 nmol/24 hr) or length of the luteal phase (≥10 days), in that the risk of conception for any one ovulation is only 25% or less, and the guidelines therefore provided even greater protection than predicted.

In Canada, the mean first estimated day of ovulation as determined from the urinary estrogen peak was 227.6 ± 90.9 days, whereas the first ovulation with an adequate luteal phase was at 264.8 ± 108 days. The range was wide, 60 to 405 and 94 to 494 days, respectively. The Australian data are not complete, but with the preliminary data as a base, the first ovulation occurred between 76 and 455 days, at a mean of 237 days. In the Sydney study the earliest ovulation with an apparently adequate and normal-length luteal phase was

^{*}Range in parentheses.

Ask the mother:



- (1) It must be noted that these guidelines are conservative. Women who follow these guidelines after six months postpartum, or who have experienced only one vaginal bleed, may still have some decreased fertility if the recommended optimal breastfeeding behaviors are followed. Furthermore, in many areas of the world, women may breastfeed for 18-24 months and remain amenorrheic for 12 months or more. These women may remain infertile for 12-15 months postpartum.
- . (2) Spotting that occurs during the first 56 days is not considered to be menses.
 - (3) "Full" breastfeeding includes exclusive or almost exclusive breastfeeding (occasional tastes of ritual foods or water), day and night, according to recommendations. "Nearly full" breastfeeding means that occasional non-breast feeds are given.

Fig. 1. Use of LAM for child spacing during the first 6 postpartum months.

on day 76, in a woman who weaned her baby on postpartum day 63. These findings suggest that individual women should begin to use an alternative method of family planning, including NFP, once they begin supplements after 6 months during lactational amenorrhea, if they wish to avoid pregnancy. However, it is obvious from the data that many women will remain amenor-rheic and/or anovulatory well beyond 6 months or beyond the introduction of supplements (see Fig. 1).

The shortest intervals between the Bellagio guidelines and the first ovulation in the Australian group were 8 and 15 days in two subjects who resumed bleeding while fully breastfeeding. As indicated above, 6 days of spotting heralded the ovulation for one woman and 1 day of bleeding and 3 days of spotting for another. The mean interval between the postpartum days by LAM to the first ovulation was 103 days (range, 8 to 272) and to the first normal/adequate ovulation, 134 days (range, 11 to 311).

The appropriate use of LAM results in a 2% pregnancy rate. The use of NFP, particularly the cervical mucus methods, may assist a woman to recognize the pending follicular development and ovulation. In three Australian studies of cervical mucus observations during breastfeeding, no pregnancies occurred before the first bleed, whether the bleed occurred during full breastfeeding, during partial breastfeeding, or after weaning. Studies in Chile also indicate high effective-

Table II. Mean number of days ± SD (range) to first bleed and ovulation and provided by Bellagio guidelines (LAM) protection (Family Health International/International Institute for Studies in Natural Family Planning Multicentre Breastfeeding/Natural Family Planning Study)

Country	N	First bleed	First ovulation	Bellagio protection
Australia	25	230 ± 112.9 (84-465)*	237 ± 105.4 (76†-455)	134 ± 38.6 (55-183)
Canada	25	221 ± 88.5 (68-356)	228 ± 91 (60-405)	132 ± 36.4 $(64-183)$
United Kingdom	25	199 ± 114.5 (71-470)	(22 222)	112 ± 34.4 (54-183)

^{*}Range in parentheses.

Table III. Comparison of number of days of abstinence required by NFP methods with number of days protection provided by Bellagio guidelines or LAM

	Pollagio hyptaction	Abstinence during Bellagio protection				
Country	Bellagio protection (mean no. of days)*	Mean no. of days†	Mean % of days‡			
Australia	134	17	11.7			
	(55-183)§	(0-69)	(0-37.7)			
Canada	132	25	17.3			
	(64-183)	(0-97)	(0-58.4)			
England	112	21	17.6			
U	(54-183)	(0-60)	(0-43.4)			
TOTAL	126	`21 ´	15.5			
	(54-183)	. (0-97)	(0-58.4)			

^{*}Includes the first 42 postpartum days for all women.

ness of the ovulation method, particularly during full breastfeeding and during the first 6 months.²³

One of the disadvantages of using cervical mucus observations to predict ovulation may be the inability to recognize the infertile days because of the continuous and confusing mucus patterns that result in prolonged abstinence. 14. 24.26 An interesting comparison of the number of days of abstinence imposed by NFP observations with those imposed by LAM can be made by using the data from the multicenter study described in the article by Kennedy et al. in this issue (Table III).

As shown in Table II, the number of days of protection provided by the Bellagio guidelines was on average 126 days (range 55 to 183). The calculated number of days of abstinence that would have been required based on mucus observations (and basal body temperature in some cases) during this period of LAM use ranged from 0 to 97 days (mean, 21 days), with up to 58.4% of these days actually requiring abstinence (with a possible maximum of 69%). By using NFP, 20 of 75 couples would have had to abstain for more than 20% of the days indicated as infertile by the LAM criteria.

By contrast, 30 of 75 couples identified less than 10% of the infertile days as requiring abstinence; their fer-

tility signs were closely related to the infertility indicated by LAM. It could be argued that the frequency of intercourse is naturally low during this time,²⁷ but this could also be due to lack of confidence in their infertility based on NFP signs, a low libido, fatigue, or other reasons. The point of the analysis is that LAM can minimize the time required for abstinence and result in a low risk of pregnancy.

Future research

Many questions remain. What are the limitations of LAM? Can the 2% risk of pregnancy with LAM be further reduced? Is spotting a warning of impending ovulation for those likely to be at risk of becoming pregnant? Can a previous lactation experience be used as a guide?

It is also important to define what constitutes full breastfeeding. Is there a minimum number of breastfeeds by day and/or by night? Is there a minimum duration of a suckling episode or total suckling during a day? Is there a maximum interval between feeds? Is this more important by day or by night? Is there a minimum intensity of suckling? Does supplementation and pacifier use in small amounts or after suckling af-

[†]Ovulated before first bleed but after weaning at 63 postpartum days.

[†]The maximum possible number of days of abstinence is 183 - (42 + 14) = 127 days, where 14 is the number of days required after day 42 to describe the infertile pattern.

[‡]Greatest possible proportion is 69.4%.

[§]Range in parentheses.

fect the physiologic impact? How much other food (liquid or solid), by quantity or frequency or caloric content, can be included as almost fully breastfeeding? How much do early postpartum factors in the establishment of breastfeeding affect subsequent fertility? What characteristics of the infant, the mother, and the breastfeeding patterns affect lactational infertility? Are there environmental factors that influence breastfeeding patterns or physiologic responses?

Policy questions

A number of policy questions have yet to be resolved. Should LAM be included in NFP training and teaching programs? What adjustments to teaching need to be made both to support breastfeeding as well as to initiate NFP during breastfeeding? What education and motivation of teachers are needed to increase confidence in LAM? What ancillary education programs are needed to inform teachers and clients of the factors that maximize the infertility effects of breastfeeding? What support is needed for clients to benefit from the LAM? How should NFP methods be applied after return of menstruation?

Conclusions and recommendations

The natural protection afforded by LAM should be incorporated into the teaching of NFP. It can provide couples with security in their postpartum infertility, minimize the abstinence that might otherwise be necessary as a result of confusing mucus or basal body temperature observations, and allow couples to enjoy their new infant and renew their relationship. The interface with NFP is obvious. The woman can still be aware of and observe symptoms that can alert her to the possibility of approaching fertility. More detailed observations and charting should begin at the end of the natural protection provided by LAM, and application of the appropriate method of NFP should then be made to avoid pregnancy.

The studies carried out in collaboration with Family Health International were under the direction of Kathy Kennedy and with collaborating scientists Dr. Suzanne Parenteau-Carreau, Serena, Montreal, Canada, and Dr. Anna Flynn, University of Birmingham, United Kingdom. Urinary estrogen and pregnanediol assays were carried out by Joanne Holmes and Gillian Barker under the direction of Professor James Brown at the Department of Obstetrics and Gynecology, Royal Women's Hospital, Carlton, Melbourne, Australia, and also the help of Sheila Kippley (Couple to Couple League International, Inc., Cincinnati, Ohio).

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The natural family planning—lactational amenorrhea method interface: Observations from a prospective study of breastfeeding users of natural family planning

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Methods of natural family planning are sometimes difficult for women to use during lactation. When this is so, the lactational amenorrhea method may prove useful. Researchers agree that a fully breastfeeding woman who is amenorrheic is 98% protected from pregnancy for up to 6 months after delivery. The fertility status of 74 users of natural family planning during the time they would have been protected by the lactational amenorrhea method is examined. Underlying hormonal profiles show that there was little ovarian activity during this time. Eight ovulatory events occurred during the period of protection by the lactational amenorrhea method, of which four fulfilled minimum criteria for adequacy; there were no pregnancies during this period. However, some women did report experiencing fertile mucus symptoms during this time that were often unrelated to estrogen production. Using the lactational amenorrhea method rather than natural family planning allows them to avoid unnecessary abstinence. (AM J OBSTET GYNECOL 1991;165:2020-6.)

Key words: Natural family planning, breastfeeding, lactational amenorrhea method, postpartum infertility

Lactational amenorrhea method (LAM) is the informed use of lactational amenorrhea, during full or nearly full breastfeeding, as a means of avoiding pregnancy for up to 6 months after delivery. It is based on the Bellagio Consensus Statement, a summary of 13 studies from eight countries that concluded that "breastfeeding provides more than 98% protection from pregnancy during the first 6 months postpartum if the mother is 'fully' or nearly fully breastfeeding and has not yet experienced vaginal bleeding after the 56th day postpartum."^{2,3}

Natural family planning (NFP) methods have been adapted for use during lactation and have been taught and used successfully by various NFP programs around

the world. However, unlike the usual cervical response to estrogen production in the normally cycling, non-lactating woman, cervical mucus production during lactation does not always reflect the underlying ovarian physiology. ^{4,5} During lactation, cervical mucus production is usually a consequence of estrogen secretion. However, when mucus with fertile characteristics is produced in the absence of ovarian activity and hormone production, the use of NFP during breastfeeding can be difficult. If this unexplained mucus production is irregular, establishment of a basic infertile pattern could be especially difficult and could require several weeks of abstinence following NFP rules.

The frequency of unexplained fertile-type mucus production during lactation is not known, although it was observed, with confirmation by ovarian ultrasonography, in 18 breastfeeding women, (not randomly selected),⁴ and a similar case is also described herein. Consequently, it is of interest to know whether the use of LAM for up to 6 postpartum months by experienced NFP users would be useful, especially in terms of the amount of postpartum abstinence recommended by NFP that could be averted by using LAM.

A study of the use of the symptothermal method (STM) by breastfeeding women was conducted through Serena, Montreal, Canada; Westmead Hospital in Sydney, Australia; and the Birmingham Maternity Hospital in England. The purpose of the study was to evaluate the use of STM by breastfeeding women to learn

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Table I. Duration of natural protection, days of recommended abstinence, and days with fertile mucus	,
symptoms during LAM protection ($N = 25$ per center)	

	Montreal		Sydney		Birmingham		Total	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
No. of days of LAM protection	132	64-183	134	55-183	112	54-183	126	54-183
No. of days during LAM protection in which STM recommended abstinence	25	0-97	17	0-69	21	0-60	21	0-97
% of days during LAM protection in which STM recommended abstinence	17%	0%-58%	12%	0%-38%	18%	0%-43%	16%	0%-58%
No. of days during LAM protection with fertile mucus symptoms	21	0-115	15	0-65	16	0-68	17	0-115
% of days during LAM protection with fertile symptoms	15%	0%-67%	10%	0%-38%	14%	0%-58%	13%	0%-67%

whether it is biologically reasonable to use STM during breastfeeding to predict the recovery of fertility, and to determine whether NFP symptoms are related to the hormonal pattern that is presumed to cause them. This study permitted three types of observations related to the interface between NFP methods and LAM: (1) a description of the mucus symptoms and estrogen production during the period of natural protection as defined by LAM; (2) an example of the kind of woman who would benefit by substituting LAM for NFP in the first 6 postpartum months; and (3) a description of the single unplanned pregnancy and how it is related to both LAM and STM.

Method

Twenty-five experienced STM users were recruited in each center. The women were normal and healthy, as were their babies; they intended to use STM to prevent pregnancy and to breastfeed for at least 6 months. Each day they completed a detailed two-page diary sheet that covered questions about infant feeding and about their natural fertility symptoms (qualities of cervical mucus, basal body temperature, and, for some women, the position of the cervix). The women also collected a timed urine sample every day from which levels of estrogen⁶ and progesterone⁷ metabolites were measured and from which the estimated day of peak estrogen production was determined by the laboratory at the Royal Womens' Hospital of the University of Melbourne, Australia. One woman was not included from parts of the analysis because there were too many missing urine samples to draw conclusions about her fertility status.

"Ovulation" is said to have occurred if a rise in total urinary estrogen above 10 μ g/24 hr is seen, followed by a rise in pregnanediol glucuronide to at least 4.5 μ g/24 hr. The ovulation was called "inadequate" if pregnanediol glucuronide excretion was low (\geq 4.5 but <9.0 μ g/24 hr) and/or if the luteal phase lasted less than 10 days. If pregnanediol excretion was greater (\geq 9.0 μ g/24 hr) and if the luteal phase was 10 days or

longer, the ovulation was called "adequate" (or sometimes "possibly adequate" because the criteria for adequacy are the bare minimum values associated with normal cycling).

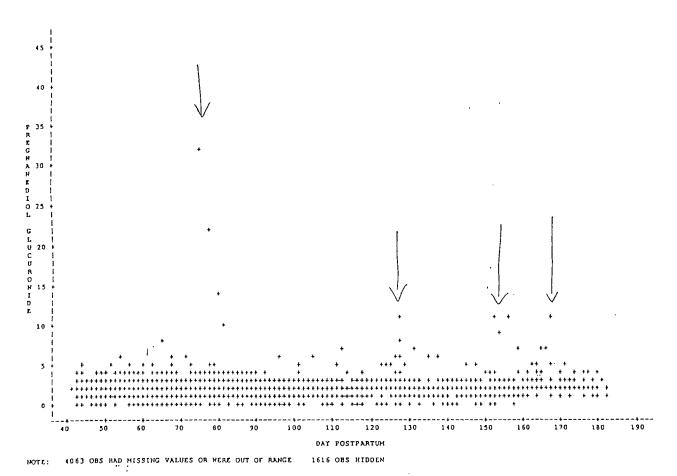
Follicular development is considered to be present when the concentration of total urinary estrogens is $\geq 15 \,\mu g/24$ hr, particularly when it is part of a rising pattern. (Concentrations between 10 and 15 $\,\mu g/24$ hr could possibly reflect follicular activity when they are part of a pattern of increasing estrogen concentration, and would be expected in only a minority of women with very low baseline levels of estrogen.)

Results and explanation

The duration of natural protection from LAM. According to LAM a breastfeeding woman can experience up to 183 days (6 months) of natural protection from pregnancy as long as she remains amenorrheic and breastfeeds her baby without supplements. When the LAM guidelines were applied in this study, the period of natural protection ranged from 54 to 183 days, averaging 4.1 months (Table I). In each study center some women did experience the maximum number of days of LAM protection (four, six, and two women in Montreal, Sydney, and Birmingham, respectively). The women in all three centers usually began supplements by the end of the sixth month, and the duration of LAM protection ended at the commencement of supplementation in 16, 13, and 16 women in Montreal, Sydney, and Birmingham, respectively.

Abstinence by use of STM during LAM protection. During this period of natural protection according to LAM, days 0 to 97 would have been days in which abstinence was recommended if the STM was used correctly. Expressed in percents, during 0% to 58% of the time that was infertile according to LAM, abstinence was recommended according to STM (Table I).

Mucus symptoms during LAM protection. We also see in Table I that from 0% to 67% of the days of natural LAM protection are days on which fertile mucus symptoms occurred. Some of the women reported



The Lactational Amenorrhea Method (LAM) is a natural method of family planning for breastfeeding women. It should be incorporated into Natural Family Planning (NFP) programs and teaching.

Fig. 1. Distribution of number of days between end of Bellagio protection (LAM) and the first day of ovulation as estimated from urinary pregnanediol levels. (Note that ovulation may not have been normal ovulation with adequate luteal phase.) (Illustration provided by Georgetown University.)

no fertile mucus during this period. Some of the reports of fertile-type mucus were accurate reflections of the underlying ovarian activity, and they preceded the first ovulations, albeit usually inadequate ones. In other cases, however, the mucus symptoms were deceptive, portraying fertility when there was in reality no estrogen production.⁴ In this situation NFP can be very difficult to use (see following case description).

In Fig. 1 progesterone production—the hormonal consequence of ovulation—is reported as micromoles of urinary pregnanediol per 24 hours. The four potentially adequate cycles can be seen easily as the four episodes in which pregnanediol rose above the minimum value associated with normal luteinization (9 µmol/24 hr).

Only one of these four potentially adequate episodes (the first) reflects a significant amount of pregnanediol excretion. In this case, during the 2 weeks immediately before ovulation, the woman breastfed her baby as seldom as five times in a 24-hour period, with interepisode

intervals as long as 9½ hours. She also reported that the baby had colic and that the breastfeeds were therefore brief. During about half of this period, she breastfed only during the day and not at all at night. She had also given her baby a bottle of milk or formula for 3 days in the week before ovulation. Since she had not yet given the baby supplemental food every day for 7 consecutive days, she was not yet categorized in this study as "supplementing"; however, this pattern would be considered less than full breastfeeding.

The three remaining potentially adequate cycles in the lower panel of Fig. I were each characterized by a 10-day luteal phase. The likelihood is very low that such a brief luteal phase could have characterized a cycle capable of sustaining a pregnancy. In Fig. 2 these three cyles with 10-day luteal phases are counted as "adequate" in an inverse life table depiction of the percentages ovulatory during the period of LAM protection because there is a slim chance that the event was fully fertile.

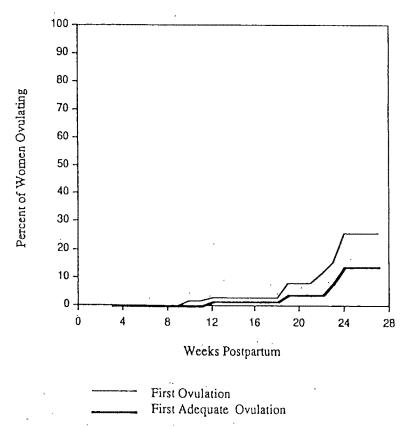


Fig. 2. Time to first evidence of ovulation and first adequate ovulation during LAM protection in all three centers, N = 74.

Altogether, eight out of 74 women for whom hormone data are available (11%) experienced symptoms of ovulation. In four of these cases the ovulation could possibly have been adequate (5%), although in only one case was the luteal phase greater than 10 days (1%). The life table rates for women experiencing the first ovulation by the sixth month of LAM protection (0.257) and the first possible adequate ovulation (0.139) are higher than the absolute percents mentioned above (11% and 5%) because most women were censored from the life table during the fifth and sixth postpartum months; at this point they were regularly supplementing the baby's breastmilk diet with other foods and therefore had ceased to be protected by LAM because they were no longer nearly fully breastfeeding.

To estimate the probability of pregnancy, both the absolute and life table percentages of women ovulating must be multiplied by a factor of 0.25 or lower. Since in even normally cycling, nonlactating women, no more than 25% will conceive during any given cycle, 8-10 the maximum 6-month life table pregnancy rate falls between 1.25% and 6.23%. It would be prudent, however, to remember that only one ovulation was characterized by strong evidence that it was normal; therefore we would expect a much lower pregnancy rate than this range suggests.

What kind of STM user would most benefit by using LAM? It seems intuitively obvious that any woman who is knowledgeable about LAM, who plans to exclusively breastfeed her baby for at least 6 months, and who makes an informed choice to use LAM is a good candidate for the method. We would like to give a cautionary note; if her previous breastfeeding experience reflects an early return of fertility, she may wish to take this into account in her informed choice. STM users, particularly those for whom STM rules recommend a great deal of abstinence even during amenorrhea and exclusive breastfeeding, might be especially happy to substitute LAM for STM for 6 months. For example, one woman in the Montreal center contributed most of the reported days of fertile type mucus, and she was definitely infertile during her entire 6 months of LAM protection. Fig. 3 displays her mucus symptoms and her estrogen profile for this period. This woman experienced persistent mucus, which she, as an experienced NFP user, perceived to be fertile, whereas her estrogen production remained persistently flat. She may have been an ideal candidate for LAM, since the proper use of STM required abstinence for more than a third of these days.

The unplanned pregnancy vis-à-vis LAM and STM. This study was not designed to yield an effectiveness

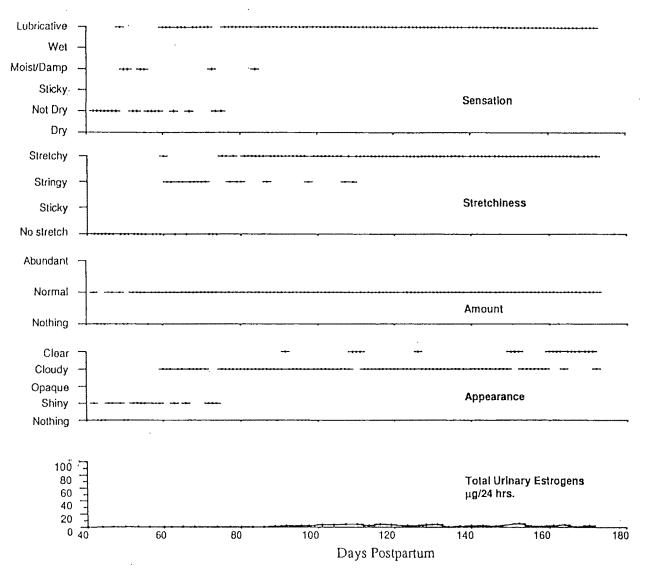


Fig. 3. Mucus symptoms and estrogen levels for mother No. 22, Canadian center. (Illustration provided by Georgetown University.)

rate for either LAM or STM. The women in the study were actively using STM as it is taught by their respective centers. One unplanned pregnancy occurred in a woman at the Montreal center at the end of her sixth postpartum month during full breastfeeding (Fig. 4). At the estimated time of the conception, she was breastfeeding an average of 10 times in 24 hours. She was not, however, still amenorrheic and, hence, no longer using LAM. She had had two bleeding episodes and an "inadequate" ovulation before the cycle in which she conceived.

The day of peak estrogen excretion (the estimated day of conception) was postpartum day 182. She had had intercourse 5 days previously, during a time when she did not report fertile-type mucus (it was described as sticky and shiny), and the woman did not believe herself to be fertile. Fertile-type mucus appeared 2 days after the day of sexual relations and lasted for 2 days.

The woman considered herself fertile for 3 days (the 2 days of fertile mucus plus 1 day). The conception probably occurred the day after that. Correct use of STM recommended abstinence from day 169 through day 184. Although her symptoms were not necessarily "fertile," they were different from—and elevated above—her basic infertile pattern. This was particularly significant because she had already experienced two bleeding episodes, which should have motivated her to attend to the rules for abstinence.

There are several points of note regarding this pregnancy and LAM. Since this woman had experienced two bleeding episodes before the conception, it did not occur during the period of LAM protection, since vaginal bleeding episodes are nearly always the consequence of some form of ovarian activity (e.g., ovulation, inadequate ovulation, estrogen withdrawal because of the collapsed follicle). The high level of natural pro-

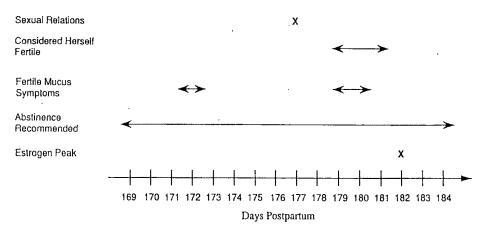


Fig. 4. Unintended pregnancy in mother No. 2, Canadian center.

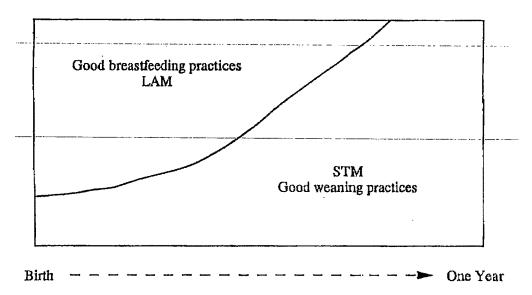


Fig. 5. Suggested emphasis in NFP-breastfeeding counseling. (Illustration provided by Georgetown University.)

tection provided by LAM has ceased once lactational amenorrhea has ended. For NFP users who use LAM during the first 6 months, the occurrence of bleeding is a warning to be taken seriously. Perhaps if this woman had appreciated the significance of her bleeding episodes, she would have been more attentive to her symptoms and applied the rule more exactly.

Comment

These data from the NFP and breastfeeding study suggest that the option to use LAM during the first 6 postpartum months may be helpful to some couples and appears to pose no additional risk of pregnancy in terms of the rates of adequate ovulation during the period of LAM protection. NFP programs would need to incorporate the teaching of good breastfeeding skills and principles to make this effort successful.

This analysis implies at least three potential programmatic recommendations. These recommendations

are speculative but would appear to be logical corollaries of the findings reported here in the context of modern NFP programs. The first is that the use of LAM among NFP users should be encouraged when possible. There is a risk of pregnancy, but it appears to be no greater than the risk associated with using other NFP methods. To their benefit, couples could avoid unnecessary abstinence but, perhaps more important, could be relieved of the possible frustrations associated with trying to identify a dynamic basic infertile pattern.

Second, couples should be urged to pay very serious attention to the return of menses or bleeding. Because the STM rules require couples to abstain until the observed postovulatory phase in the first three cycles, there can be a considerable time of abstinence, but it may be preceded by an abstinence-free phase if LAM is used.

Third, in postpartum NFP programs good breastfeeding skills or principles should be taught. By helping women to be successful breastfeeders, NFP programs may also observe parents who are more confident regarding both the care and nourishment of their child and their protection from pregnancy, longer natural durations of lactational infertility, fewer frustrations for couples and teachers, and greater professional fulfillment for teachers. All involved may also be pleased to know that they are reducing their child's risk of many infections and some chronic diseases. These are some potential benefits that NFP programs may consider measuring and cultivating as they develop programs and strategies for testing the integration of LAM and NFP.

Our support of breastfeeding families might take a profile suggested in the continuum in Fig. 5. At first, greater educational effort could be focused on breastfeeding skills (such as how to help a baby attach to the breast) and on breastfeeding principles (such as feeding the baby frequently day and night, avoiding bottles and pacifiers, and the like). Also, of course, an overview of how to use NFP in the postpartum period could be given so that LAM rules can be followed as soon as LAM protection expires. Gradually the couple will need to know about good weaning practices and given detailed information about how to handle at least the first three menstrual cycles. NFP instructors would need to acquire more skill and more knowledge, but these would be germane to teaching couples to be in charge of their own natural infertility and recovery of fertility, since infant feeding practices are directly related to postpartum fertility.

We gratefully acknowledge the help of the technical staff responsible for conducting the hormone analyses and the NFP teachers who conducted the field work, namely, Dr. Meg Smith, Joanne Holmes, Gillian Barker, Denise La Flamme, Lise Trepanier, Gail Byrne, and Anne McCarthy. Finally, our deep gratitude goes to the mothers who persevered in providing daily diary sheets and urine samples over months and years.

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Ultrasonographic patterns of ovarian activity during breastfeeding

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In this study, ultrasonography was used to detect follicular activity in lactating women, and these findings were related to the underlying hormonal profiles and to the mucus symptom. A number of different patterns of follicular development were seen before the women returned to normal fertile cycles during the period that was previously considered to be characterized by ovarian quiescence. Some of the transitory patterns of follicular activity were reflected in rising hormone levels and patterns of fertile mucus that were sometimes confusing for these lactating women who were using natural family planning. (AM J OBSTET GYNECOL 1991;165:2027-31.)

Key words: Natural family planning, lactating women, ultrasonography, follicular growth, cervical mucus

Ultrasonographic examination is a well-established technique for monitoring follicular and ovarian activity in both spontaneous and induced ovulatory cycles, and there are several publications that describe the patterns of follicular growth and development that are found in different clinical situations. ¹⁻⁴ In contrast, ovarian and follicular activity in women who experience returning fertility during and after lactation is less well documented. ^{5, 6} Shaaban et al. ⁶ have studied lactating Egyptian women between 6 and 12 months after delivery, but many of these women were no longer amenorrheic. Flynn et al. ⁵ observed only two women during weaning.

Hormone profiles have been used to describe the endocrine and gonadotropin activity in the hypothalamic-pituitary-ovarian axis. These studies have contributed to an understanding of the underlying physiology that is associated with lactation infertility. To ther researchers have studied the relationship between ovarian hormone production and women's observations of cervical mucus and/or basal body tem-

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Reprint requests: Anna M. Flynn, MD, Natural Family Planning Centre, Department of Obstetrics and Gynecology, Birmingham Maternity Hospital, Queen Elizabeth Medical Centre, Edgbaston, Birmingham B15 2TG, United Kingdom. 6/0/34248 tation, breastfeeding women often experience fertiletype mucus symptoms at times that are unrelated to ovulation. ¹² Consequently, such women can have difficulty in using natural family planning at this time without on the one hand, an undue (and often unnecesssary) amount of sexual abstinence or on the other hand, exposure to the risk of an unplanned pregnancy. The aim of this project was to (1) observe (through serial ultrasonography) and describe the different pat-

perature. 10-13 Although a peak mucus symptom is very

frequently associated with ovulation even during lac-

The aim of this project was to (1) observe (through serial ultrasonography) and describe the different patterns of follicular growth and development before normal fertile cycles become established in the lactating mother and (2) to relate these patterns to the underlying hormonal profiles and to the cervical mucus symptoms.

Methods

Beginning at 6 weeks after delivery, 18 lactating women collected daily time samples of urine, which were stored frozen at 17° C and subsequently analyzed for total urinary estrogen¹⁴ and pregnanediol¹⁵ concentrations. Each women completed a daily record of mucus observations and basal body temperature. Each woman also kept a daily record of the number of breast feeds, bottle feeds, and/or solids given to the baby both over the day and during the night.

Follicular tracking was carried out with a G.E. RT 3000 real-time scanner with a 5 MHz phased array sector probe, each woman who was examined had a full bladder, and the abdominal scanning technique was used. Scanning was begun during the twelfth week and was continued at weekly intervals until the ultrasonographer noted developing activity and/or the lactating woman found that her clinical indicators (mucus, cervix, and basal body temperature) suggested that she

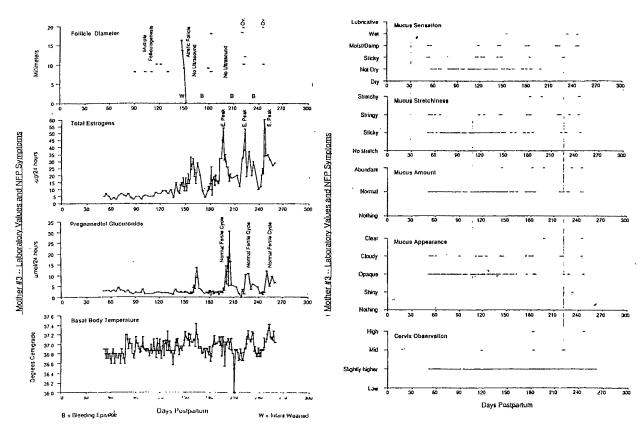


Fig. 1. Mother No. 3: Laboratory values and natural family planning symptoms. (Illustration provided by Georgetown University.)

Table I. Patterns of follicular development observed during lactation

Multiple folliculogenesis
Unruptured follicles
Luteinized unruptured follicle
Atretic follicle
Persistent unruptured follicle
Recurrent large follicles which ruptured
"Delayed" ovulation
Normal follicular growth (fertile cycles)

was potentially fertile. When either one or both of these conditions were present, follicular tracking was carried out at more frequent intervals until the ovarian activity decreased or the follicle ruptured whether or not the ovulatory event occurred.

In the same manner that was used to detect ovulation of normal reproductive cycles in nonlactating women, the ultrasonographer looked for follicular rupture with possible ovulation and the following indicators of fertility: the development of a follicle with a maximum diameter of 18 to 25 mm over a period of 3 to 6 days and its subsequent rupture or decrease in volume by 50% over 24 hours; the presence of fluid in the pouch

of Douglas, if seen, around the time of rupture; thickening of the endometrium, if seen; and the presence of a corpus luteum within 24 to 48 hours after follicular rupture. All data collection procedures were followed until after the second normal postpartum ovulatory cycle.

Results

Table I outlines the different patterns of follicular development that were found in the women who were studied. No consistent serial pattern emerged; in some women, multiple folliculogenesis was quickly followed by normal fertile cycles, whereas in others, several transitional patterns occurred before fertility became established. Ultrasonographic records were examined for episodes of follicular development and ovarian activity. Each episode was categorized by the pattern presented, some of which are illustrated in Figs. 1, 2, and 3.

Multiple folliculogenesis. Multiple folliculogenesis was a very common finding during the early stages of returning fertility in almost all of the lactating women who were studied. In these women, several small follicles with a typical diameter of approximately 8 mm were observed in one or both ovaries. The number varied but ranged between 3 and 20. Their appearance

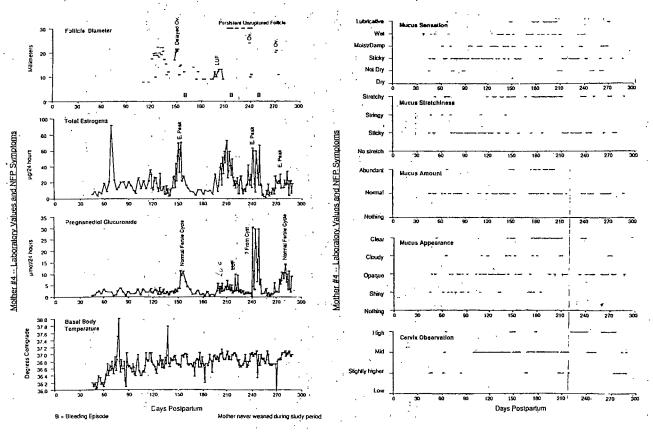


Fig. 2. Mother No. 4: Laboratory values and natural family planning symptoms. (Illustration provided by Georgetown University.)

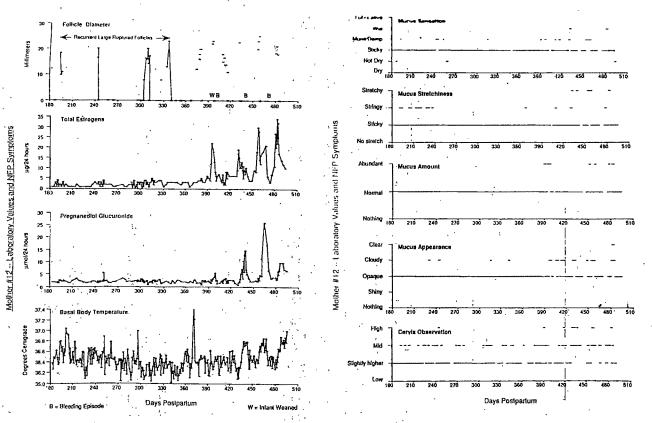


Fig. 3. Mother No. 12: Laboratory values and natural family planning symptoms. (Illustration provided by Georgetown University.)

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was episodic and there were intra- and interwoman variations in the duration of these multiple follicles.

In general, these follicles caused no increase in the concentrations of estrogen and progesterone metabolites, and consequently, the women observed no increase or change in the cervical mucus and patterns (Fig. 1). Eventually, in all cases, one follicle became dominant and the others regressed.

Unruptured follicles. Three types of unruptured follicles were observed: the luteinized unruptured follicle, the atretic follicle, and the persistent follicle (cyst). In the first two situations the follicle grew slowly and reached a maximum diameter that was somewhat smaller than normal. The follicle did not rupture but slowly decreased in size over the subsequent days and finally disappeared.

In the luteinized unruptured follicle, estrogen and pregnanediol levels increased without reaching the concentrations that are found in the normal ovulatory cycle (Fig. 2). In the atretic follicle there was no increase in the pregnanediol concentrations (Fig. 1).

In the persistent unruptured follicle, ultrasonographic findings showed the growth and development of what appeared to be a normal follicle. Development continued, and in some cases the follicle increased in size to a diameter of 30 mm, which was maintained for weeks until it finally ruptured or regressed. Some of these large persistent follicles were associated with estrogen excretion as well as the production of fertiletype mucus for long periods and with a pattern that was impossible to distinguish from that of the normal fertile phase (Fig. 2).

Recurrent persistent follicles. We found this pattern in only one mother who intensively breastfed her infant for almost a year. From approximately 6 months after delivery, a follicle developed that reached a maximum diameter of at least 18 mm over 1 to 3 days and then ruptured within 24 hours. Initially, these episodes occurred at 60- to 90-day intervals, but later in lactation they recurred at intervals of 30 days. These follicles did not produce any increase in ovarian hormones nor were they accompanied by any clinical signs of fertility or vaginal bleeding until the infant was weaned at 57 weeks when there was a sudden resumption of fertile cycles (Fig. 3).

Delayed ovulation. In this pattern, the leading follicle developed normally to its maximum diameter at which time an estrogen peak was observed in the urine. However, instead of rupturing as expected, the follicle maintained its size for 2 to 3 days before it ruptured. Hormone concentrations and the length of the luteal phase were similar to those for the normal fertile cycle and menstruation followed (Fig. 2).

Comment

Until recently, the period of lactational amenorrhea had been considered largely a time of ovarian quiescence. Serial ultrasonographic examination of the ovary in lactating women showed that the ovarian activity can begin, and several different patterns of follicular activity can occur before full fertile cyclicity becomes established. In some of these transitory stages, concentrations of total urinary estrogens and pregnanediol were increased, which caused cervical mucus patterns that are consistent with fertility. This was confusing for the lactating women who were using natural family planning methods to avoid a pregnancy. These results suggest the limitation of the mucus symptom in predicting a truly fertile cycle in the lactating woman.

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Ovulation method use during breastfeeding: Is there increased risk of unplanned pregnancy?

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Data from two prospective studies of the ovulation method were used to assess pregnancy rates and users' fertility-related behaviors among breastfeeding women. The rate of unplanned pregnancy was <1% during the first 6 months of lactational amenorrhea. However, the unplanned pregnancy rate was elevated among breastfeeders during the months after menses return compared with the pregnancy rate during nonlactating cycles. Rates were also elevated at the time when infant feeding supplementation was started. This increase in unplanned pregnancies was not directly attributable to nonadherence to the ovulation method rules; there was some indication that adherence to the rules actually may be increased during those months. Therefore, special emphasis on both the need for improved breastfeeding support to delay menses return and the increased potential for method failure among new users during this period of time should be incorporated into ovulation method training and support programs. (AM J OBSTET GYNECOL 1991;165:2031-6.)

Key words: Ovulation method, breastfeeding, Kenya, Chile, lactational amenorrhea, unplanned prengancy, supplemental feedings

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Data from two prospective studies of the ovulation method (OM) were analyzed to assess pregnancy occurrence and related behaviors. The first was carried out in Nyahururu, Kenya. It was originally designed to assess the cost-effectiveness of OM and the impact of different intensities of training. The second was in Santiago, Chile, and was designed to study the efficacy of the OM during breastfeeding. The purpose of this article is to illustrate that (1) special times during breastfeeding, especially the months of lactational menses and time after the commencement of supplemental infant feedings, can be associated with increased preg-

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Table I. Nyahurur	ru study: Cumula	tive pregnancy	rates per 100	OM users by	lactation status an	d
age group						

			Lacte	ating					Not la	ctating		
	(n =	ll 205)	<30 (n =	0 yr 105)	≥30 (n =		(n =		<30 (n =	9 yr 55)	≥ 3 (n =	
Cycle No.	%	SE	%	SE	%	SE	%	SE	%	SE	%	SE
3	0.7	0.5	1.3	0.9	0		1.7	1.0	1.8	1.7	1.7	1.1
6	6.0	1.4	8.9	2.3	2.8	1.4	7.5	2.0	7.0	3.4	7.7	2.5
9	3.3	2.0	15.9	3.0	10.4	2.6	11.2	2.4	10.8	4.2	11.4	3.0
12	18.5	2.4	19.6	3.4	17.0	3.4	14.1	2.7	13.3	4.7	14.5	3.4

Lactation status and age group are those at time of first cycle.

nancy rates among OM users; (2) this phenomenon is not a result of laxity in method adherence on the part of the users; and (3) because breastfeeding is the norm in most areas of the world, and because international support for optimal breastfeeding practices will be increasing, it is necessary for OM trainers to be aware of the above facts and be skilled in the appropriate introduction and use of OM during breastfeeding.

Methods: Nyahururu

Details of the Nyahururu study have been presented elsewhere. This was an operations research study in which training protocols of different lengths were introduced and client data and charts were collected monthly from the time the couple attended the first teaching session to learn the method. The data were sent to the Johns Hopkins University for analysis.

Data included basic information about each woman, such as teaching method, age, and other background information, along with data for charted cycles. Cycle is defined as the period from first day of one menstrual period to the first day of the subsequent period. In nonmenstruating women, the cycle is the period of time charted between interviews, approximately 30 days. After exclusion of women who were sterilized (seven), unable to maintain charts (nine), or not trying to avoid pregnancy, the data file contained information for 521 natural family planning (NFP) users from 42 sites near Nyahururu who entered the project between September 1983 and November 1986. Data collection for this project was carefully supervised by the project director and was generally of good quality, although there were some problems maintaining uniformity in the data collection procedures over time.

Analyses included frequencies and means of major variables, as well as descriptive presentation. For this article the major analyses included the development of cumulative 12-month life tables with standard errors, logistic regression with use of the PHGLM package, which takes into account censored data, and descriptive tables. All statements of statistical significance are based on p < 0.05 in a two-tailed test of significance.

Results: Nyahururu

Among the predominantly rural and Catholic population of Nyahururu, the unplanned cumulative pregnancy rate was about 22 per 100 women at 12 cycles/mo. Fifty-nine percent were lactating and 47% were amenorrheic at entry. Because a lower rate of pregnancy had been expected among lactating women, the population was divided into those who were lactating at program entry and those who were not. The pregnancy rate among women who were lactating at entry and continued lactating through a first menses was 18.5%, but that among women who were not lactating at entry was only 14.1% (Table I). This is not statistically significantly different. Although numbers were small, the highest rate of pregnancy was among those who were lactating at entry and never menstruated. This trend was unexpected; a lower rate of pregnancy had been expected among lactating women.

Maternal age and stated pregnancy intention had no influence on the pregnancy rate after lactational status was controlled. Among lactating women younger than 30 years of age, the pregnancy rate at 12 months was 19.6% whereas among the older lactating women the pregnancy rate was 17.0%, which is not significantly different. Among the nonlactating women the 12-month pregnancy rates were 13.3% for the younger women and 14.5% for the older women, also not statistically significant (Table I).

Pregnancy rates after menses return were also assessed. Entry into this life table occurred at the first recorded menstrual cycle and was controlled for both lactational status and maternal age (Table II). The pregnancy rate for the 12 cycles after menses return among women whose menses began during lactation was 36.5% for the younger women and 32.9% for the older women. The rate among menstruating, nonlactating women from the time of the first recorded menses was approximately 13% for all age groups. Within lactation status, no significant differences were seen, but the difference between the pregnancy rates of cycling lactating women and cycling nonlactating women was highly significant (p < 0.0001).

Table II. Nyahururu study: Cumulative pregnancy rates per 100 OM users after resumption of mense	s,
by lactation status and age group	

		Lact	ating			Not la	ctating		
	<30 (n =			$\geq 30 \text{ yr}$ $(n = 100)$		<30 yr $ (n = 55)$		$\geq 30 \text{ yr} \\ (n = 108)$	
Cycle No.	%	SE	%	SE	%	SE	%	SE	
3	11.8	3.2	7.2	2.6	3.6	2.5	3.7	1.8	
6	26.8	4.6	13.1	3.6	9.1	3.9	9.5	2.8	
9	36.6	5.5	27.1	5.2	13.6	4.8	12.6	3.3	
12	36.6	5.5	32.9	5.8	13.6	4.8	12.6	3.3	

Lactation status and age are those at time of first cycle. Life table entry at first charted cycle with menses.

Table III. Nyahururu study: Percentage of cycles with intercourse occurring within each phase of cycle, by lactation status and by whether unplanned pregnancy occurred

	U	nplanned pregna	псу		No pregnancy	•
	Prefertile	Fertile	Postfertile	Prefertile	Fertile	Postfertile
Lactating	59.7	5.4	28.7	65.8	5.8	15.8
Nonlactating	55.4	18.5	59.8	39.1	7.4	60.2

Occurrence of intercourse during fertile days was much higher in cycles of nonlactating women who become pregnant compared with those who did not become pregnant. However, intercourse activity was not elevated in cycles of lactating women who become pregnant.

The pregnancy rates for the first 3 to 6 months after menses return were extraordinarily high among lactating women, approximately 12% in the younger women and 7% in the older women. This age group difference also may be caused in part by a difference in intensity of breastfeeding practiced by the different age cohorts, because it disappears by the end of the first year of lactational menses. Furthermore, by logistic regression analysis, the major single predictive variable for unplanned pregnancy was lactational status at entry (p < 0.0007). The only other variable predictive of unplanned pregnancy was entry into the study relatively early postpartum (p < 0.035).

The next series of analyses was concerned with whether the women who had unplanned pregnancy during lactational menses were ignoring the rules of the method. An analysis of the percentage of women who had intercourse during the prefertile, fertile, and postfertile periods was undertaken. These results were then separated according to whether the women had had an unplanned pregnancy (Table III). Among lactating women there was little difference in timing of intercourse between those who did and did not have an unplanned pregnancy. The percentage who reported intercourse during the fertile period was 5.4% for those with an unplanned pregnancy, and 5.8% among those who did not have a pregnancy. There was a higher rate of recorded postfertile-period intercourse (28.7%) among those who had an unplanned pregnancy compared with those who did not (15.8%).

Table IV. Santiago study: Six-month cumulative life table unplanned pregnancy rates among OM users

Subset	Percent	SE
All	3.5	1.0
Amenorrheic*	1.2	0.7
Menstruating	8.2	2.7
Full breastfeeding*	1.2	1.6
Any breastfeeding	2.3	1.8
No breastfeeding	8.2	2.6
Full breastfeeding and amenorrhea	0.7	1.4
Any breastfeeding and amenorrhea	2.1	2.2

^{*}Categories significantly different at p < 0.05 level within grouping.

Among nonlactating women, however, there was a difference in the percentage reporting intercourse during the fertile days. Intercourse during the fertile days was recorded in only 7.4% of the cycles among those who did not become pregnant compared with 18.5% among those who had an unplanned pregnancy, a significant difference. The nonlactating women who had an unplanned pregnancy also reported a higher rate of intercourse in the prefertile period (55.4% vs 39.1%).

An additional analysis addressed the same issue by time postpartum. Among lactating women, there were no major trends over time except for some decrease in

Table V. Santiago	study: C	Cumulative li	ife table	pregnancy	rates after	return of menses
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	Any brea	stfeeding	1	A <i>ll</i>
Month	. %	SE	%	SE
1	1.3	1.8	1.6	0.7
3	3.4	3.4	4.8	1.2
6	19.2	2.4	8.5	1.7

Table VI. Santiago study: Linear model for variables associated with "fertility return" (as defined) duration of infertile period

Maternal age (yr) Menses	NS $p < 0.0001$
No breastfeeding at fertile symptom	NS NS
No breastfeeding two cycles before	<i>p</i> < 0.0001
Occurrence of a peak symptom	p < 0.027
No. of fertile days	NS

NS, Not significant.

total abstinence and some increase in intercourse during prefertile "dry" days. Both those who became pregnant and those who did not appear to have been adhering to the rules similarly. Among those not lactating, however, there were notable differences between those who became pregnant and those who did not. Those who became pregnant tended to have intercourse during the fertile period much more often and earlier postpartum, with relatively more intercourse in the prefertile period and relatively less intercourse in the postfertile period. The trends in total abstinence, however, were similar between lactating women and nonlactating women who became pregnant and between lactating women and nonlactating women and nonlactating women who did not become pregnant.

A third analysis on this issue assessed whether these differences might result from large differences in the number of days falling in each part of the cycle. The only finding was a slightly longer average cycle length among those who became pregnant (29 vs 28 days), which would not appear to account for the significant differences seen above.

From this we may conclude that the couples in which the woman was lactating on entry into the OM program were more likely to have an unplanned pregnancy even though they were not ignoring the rules. Rather, on average, the lactating women adhered to the rules more closely than the nonlactating, practicing abstinence on fertile days significantly more frequently.

Methods: Santiago

In Santiago a prospective study was conducted that included primarily lactating women. The data collected

Table VII. Santiago study: Number of days falling within designated sign or symptom and percent of those days in which coital activity occurred

	Total days (approximate)	% of days with coital activity
Menses	1,945	3.8
Dry	15,267	10.5
Day after intercourse	370	0.7
Fertile	1,577	0.8
Peak day	545	0.2
Infertile	5,402	10.5
Spotting	337	9.8
TOTAL	25,443	$\overline{9.0}$

N = 110.

on 378 births and postpartum OM use are reported elsewhere.² These women had a 12-month cumulative pregnancy rate of 12%.

The analyses for this article were derived primarily from the records of the 110 women for whom data were available on signs and symptoms and occurrence of intercourse by day postpartum. Data were also available on feeding (full breastfeeding, partial breastfeeding, or no breastfeeding), mother's and father's age, education, religion, number of living children, reason for accepting NFP, attitude toward an unplanned pregnancy, marital status, and previous abortion and pregnancy occurrence.

Cross tabulations were used to assess several parameters to compare usefulness of sensation versus observation for the signs and symptoms record. Student's t tests, linear modeling, and proportional hazards modeling were used to examine those variables associated with onset of a fertile episode. Tabulation and logistic regressions were used to assess variables associated with occurrence of intercourse on a prohibited day. Reported sensation signs and observation signs were compared to see whether either seemed to be associated with the identification of an appropriately timed fertile episode. Although sensation signs do occur earlier than observation in lactating women, neither was notably better at identifying a fertile period before the first menses. Sensation indicators were selected to be used for all further analyses. For these analyses, OM signs and symptoms of fertility occurring 8 to 20 days before

Table VIII. Santiago study: Percent of days for abstinence, percent of abstinence days during which intercourse occurred, and percent of nonabstinence days during which intercourse occurred, by time postpartum

Days postpartum	% of days abstinence necessary*	% of abstinence days with intercourse	% of free days with intercourse
22-90	20.2	. 4.1	13.6
91-180	33.2	3.2	20.2
181-270	41.4	2.2	20.8
271-360	45.6	4.4	22.1
361-720	47.1	9.9	31.2

^{*}Inversely related to breastfeeding.

an episode of vaginal bleeding are considered to reflect fecundability.

Results: Santiago

Life table analyses were used to explore the relationships between menstrual status, breastfeeding status, and pregnancy rates (Table IV). As expected, the cumulative pregnancy rate at 6 months was significantly lower among the amenorrheic women (1.2%) than among those whose menses had resumed (8.2%). The pattern of lactation gave a "dose response"; the more intensive the breastfeeding, the lower the occurrence of unplanned pregnancy (p < 0.05). This trend was also seen among amenorrheic women, but here the trend was not significant.

After menses returned, breastfeeding continued to have a depressive effect on unplanned pregnancy for the first 3 months. After that time, however, lactational menses again was associated with an unexpected high level of unplanned pregnancy (Table V).

Three variables were shown in a time-dependent proportional hazards model to be significant and highly predictive of fertility return. Consistent with results of other studies, the return of menses was associated with the return of fertility ($\beta = 1.6045$). The initiation of supplemental feeding, or the switch from exclusive to partial breastfeeding, also proved to be associated with fertility ($\beta = 1.6475$). A third variable, weaning or cessation of any lactation, also was associated with the return of fertility with similar strength ($\beta = 1.6527$).

Table VI confirms these findings in a linear model for association with fertility return. This model used slightly different variables (return of menses and completed weaning two cycles previously). Another variable identified with the fertility return is the occurrence of a peak symptom.

The return to fertility predicted by the proportional hazards and linear models is directly reflected in the pregnancy rates, with the important exception of higher fertility seen after 3 months of lactational menses. Because this phenomenon is not readily explained by the physiology of lactation, and because all

Table IX. Santiago study: Logistic regression model for adherence to rules for statistically significant variables

Variable	Direction of association
Maternal age 25-29 yr	+
6-9 mo postpartum	+
Father's education	+
Breastfeeding cessation	_
Mother's education	_
Religious reason	_
High receptivity	_

women in the subset of 110 were also using the mucus method to space their births, the coital behavior of this population, as recorded on the OM charts, was studied to assess whether the adherence to rules accounted for this difference in fertility. Among these breastfeeding women we found that 4% reported coital activity during menses, 10.5% on dry days, 0.7% on the day after intercourse, 0.8% on the fertile days, 0.2% on peak days, 10.5% on infertile days, and 9.8% on the spotting days (Table VII). Of interest is that this rate of intercourse, averaging about three acts per month, is lower than was found with practitioners of the lactational amenorrhea method (LAM) who were of approximately the same age and parity.3

The percentage of days that abstinence was required by the OM rules as time passed postpartum, as well as coital behavior, was calculated (Table VIII). In the first 3 months, abstinence was necessary on 20% of the days; in the second 3 months postpartum it was required on about one third of the days. In the sixth to ninth month postpartum, intercourse was prohibited on 41% of the days and later increased to 45% to 47% of the days. Correspondingly, 2% to 4% of abstinence days reportedly included coitus throughout the first postpartum year, and the percent of "free" days with a coital act increased throughout the year, beginning at about 14% in the first 3 months and increasing from 20% to 22%. After the end of the first year postpartum, the percentage of abstinence days with reported intercourse increased to 10% and the percentage of free days with reported intercourse increased to 31%.

Using LAM would have greatly decreased the number of abstinence days for these couples. It would have allowed all days up to 6 months to be free days, providing the women were fully breastfeeding their infants and were amenorrheic. Analysis of the unplanned pregnancy data from those who were breastfeeding showed that 67% of the pregnancies were user related and 25% were method related, and among those not breastfeeding 85% were user related and 15% method related.⁴

The pregnancies among lactating women primarily occurred after menses returned in the sixth to ninth month postpartum. However, the logistic regression model for adherence to rules showed that this particular variable was associated with significant adherence to the rules (Table IX).

Although some of these analyses do not have numbers large enough to achieve statistical significance, there is an apparent consistency across all of the presented analyses to illustrate that the users were attempting to adhere to the method during lactational menses but were, nonetheless, becoming pregnant.

Conclusion

There is an increased risk of unplanned pregnancy in newly trained OM users during lactational menses, and this increase does not seem to be a result of lack of adherence to the rules. In light of the global emphasis on breastfeeding and our knowledge of fertility suppression during full lactational amenorrhea, and in light of the international interest in NFP acceptability and efficacy, attention must be paid to the particular issues of support for the client during the interface between NFP and breastfeeding. We hope that future research will shed light on improved NFP method use and teaching methods for the postpartum months, as well as on improving teaching methods for the LAM/OM interface for the later months, so that couples can reliably depend on the OM during these crucial months.

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Efficacy of the symptothermal method of natural family planning in lactating women after the return of menses

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This study was designed to determine the efficacy of the symptothermal method of natural family planning during lactation. Although the method appears to give a reasonable reflection of fertility potential over time, it overlaps with the profound influence of lactation in both ovulation suppression and the delay of luteal phase adequacy. Further analysis is planned to attempt to identify those particular mucus signs and symptoms that are helpful during the transition from lactational amenorrhea to normal cycling. (AM J OBSTET GYNECOL 1991;165:2037-9.)

Key words: Natural family planning, symptothermal method, lactation, urinary luteinizing hormone

The purpose of this study was to determine the efficacy of the symptothermal method of natural family planning (NFP) in lactating women, particularly during the first three cycles after the cessation of lactation-induced amenorrhea, in a pilot study of 25 women.

Methods

A total of 28 women were screened for inclusion in the study and 25 completed the study. During the course of the study, women recorded their daily breast-feeding episodes, introduction of supplements, and their daily NFP signs and symptoms. In this case the records included basal body temperature and in all cases, bleeding or spotting episodes. The protocol was standardized so that all subjects would be recording the same set of mucus symptoms on a daily basis. All women remained in the study for an average of 13 months; the range was 9 to 17 months. All women were discontinued from the study after they had had three apparent ovulatory cycles as determined by the urinary excretion of estrone-3-glucuronide, luteinizing hormone (LH), and pregnanediol-3-glucuronide.

Results

Delineation of the day of ovulation by means of basal body temperature was compared with delineation of the day of ovulation by means of urinary LH excretion. The results among these 25 lactating women were as follows.

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For the first cycle, the shift in temperature occurred a mean of 4 days from the day of urinary LH peak +1. The proximity of the "LH peak +1" day and the basal body temperature shift improved in the subsequent three cycles to within a mean of 1 day of the urinary LH peak +1. This suggests that the initial rise in basal body temperature during the first cycle, which in 23 of 25 cases preceded the first menstruation, is considerably less accurate than in subsequent cycles and has a high false-positive rate (Fig. 1).

The length of the luteal phase in the 25 women showed a significant change over the four cycles during which they were monitored. The length was determined by the day of LH peak +1 to the day of commencement of menstruation. If the first cycle occurred within the first 6 months of lactation, the luteal phase was insufficient as determined by its length (<10 days). Only 20% of cycles, which occurred in the first 6 months postpartum had luteal phase lengths of 10 days or greater. If the first cycle occurred after 6 months of lactation, this number increased to 60% of those who had luteal sufficiency during their first cycle (Fig. 2).

To determine the first possible return to fertility, the data were analyzed relative to the postpartum day on which the first cycle occurred. Normalized to the day of the first LH peak that followed by a pregnanediol rise, 25% had the first cycle less than 200 days postpartum. An additional 25% had their first cycle between 200 and 300 days postpartum, 35% between 301 and 400 days postpartum, and 15% greater than 400 days postpartum. An analysis to relate these findings to the patterns of breastfeeding is presently underway (Fig. 3).

Comment

The use of the symptothermal method in lactating women appears to give reasonable information to these

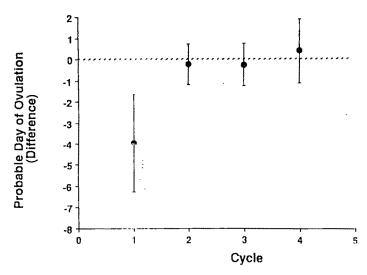


Fig. 1. Probable day of ovulation versus day of LH peak (mean \pm standard error of the mean). (Illustration provided by Georgetown University.)

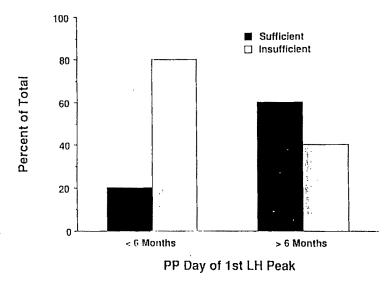


Fig. 2. Luteal phase length versus postpartum day of first LH peak. PP, Postpartum. (Illustration provided by Georgetown University.)

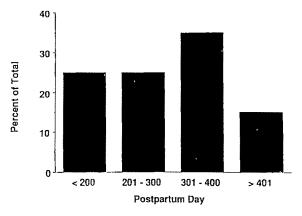


Fig. 3. Postpartum day of first LH peak. (Illustration provided by Georgetown University.)

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couples about their fertility potential. In spite of this, the influence of lactation is obviously quite profound for the first 6 months. A preliminary analysis reveals that the first cycle is frequently accompanied by an insufficient luteal phase, especially if it occurs in the first 6 months postpartum. This supports other published findings, which document lower rates of first-cycle adequacy before 6 months among lactating women.^{1,2}

In addition, the accuracy of basal body temperature

appears to be limited during the same first cycle. Further analysis of this data set should help us to understand whether there are particular mucus signs and symptoms that will help women during this early transition from amenorrhea to normal cycles.

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Santiago Breastfeeding Promotion Program: Preliminary results of an intervention study

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A prospective intervention study was undertaken in Santiago, Chile, to assess the impact of a breastfeeding promotion program and the acceptance and use of the lactational amenorrhea method for natural child spacing. The intervention study significantly increased the duration of exclusive breastfeeding and amenorrhea. In addition, the use of the lactational amenorrhea method proved highly efficacious, with an unplanned pregnancy rate of less than 0.5% by 6-month cumulative life table. Total family planning coverage at 6 months was increased in the intervention group. (AM J OBSTET GYNECOL 1991;165:2039-44.)

Key words: Lactational amenorrhea method, breastfeeding, breastfeeding promotion

The benefits of breastfeeding for both mother and child have been clearly demonstrated. Nonetheless, in the past few years in some developing countries, a decrease in the length of the breastfeeding period and changes in breastfeeding patterns have been observed. This has encouraged the development of breastfeeding promotion and support programs either hospital-based or at the community level, with diverse results. 10-14

The purpose of this intervention study (Breastfeeding Promotion Program) of urban women of Santiago, Chile, was twofold: to design and test the impact of a simple hospital-based model, which would modify

breastfeeding practices, and at the same time to design a program that could be easily replicated in other developing countries.

Moreover, the program was designed with the intention of not only increasing the number of mothers interested in breastfeeding and extending the duration of exclusive breastfeeding, but also verifying that the impact of amenorrhea caused by exclusive breastfeeding on women's fertility can be accepted and used efficaciously to delay conception. 15-17

Material and method

The Breastfeeding Promotion Program included the training of the health team, activities in the prenatal outpatient clinic, activities on the maternity ward, and an open lactation clinic.¹⁷

After gathering data on a control group of 313 mother-child pairs who followed the usual postpartum and infant feeding routines of the University Hospital of the Pontificia Universidad Católica de Chile, the Breastfeeding Promotion Program was initiated. A group of 422 mother-child pairs of urban, lower-middle class whose pregnancy, delivery, and postpartum

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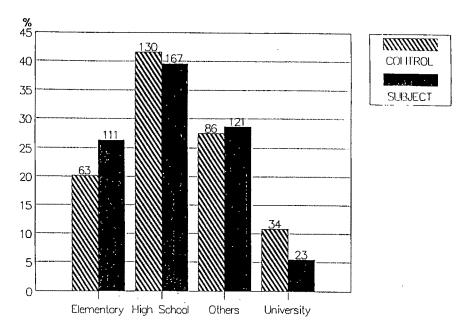


Fig. 1. Women's educational level: study group (422 cases) and control group (313 cases). (Illustration provided by Georgetown University.)

care were delivered at the hospital, were then recruited to serve as the study population.

Recruitment criteria for both groups included healthy women with no previous history of infertility; a normal breast examination; stable couple; work situation compatible with 6 months of exclusive breastfeeding; and term vaginal delivery, with a healthy child whose birthweight was over 2500 gm. The mother-child pairs in the control and study groups were followed up every 30 days until completing 180 days postpartum. The first follow-up was performed between 7 and 14 days postpartum, and this was considered the point of study entry.

During each follow-up visit, mothers were thoroughly interviewed about their breastfeeding habits, the introduction of supplementary feeding, the presence of vaginal bleeding, the frequency of sexual intercourse, and the use of family planning methods. In each follow-up the baby had a complete physical examination, and the breastfeeding technique was observed and supervised; whenever necessary, the mother was given a gynecologic examination. The infant feeding record kept by the mother for the past 24 hours before the interview was analyzed; this included the number and duration of breastfeedings during the day and night, type of supplementary feeding, time schedule, and quantity.

The mothers in the study group were requested to accept the criteria of the lactational amenorrhea method (LAM) as their child-spacing method and were advised not to use other contraceptive methods during full lactation and amenorrhea in the first 6 months postpartum. 16, 17 The analysis of the behavior of the

study group in relation to the control group included the following statistical procedures: univariate analysis with standard errors, Student t test for comparison of means, Fisher's test for 2×2 tables, and χ^2 test for tables of greater categories. In addition, life table analyses were used to assess time-related events. In the first 6 months of the program, only 3.8% of the control group and 3.0% of the study group were lost to follow-up.

Results

Population characteristics. The characteristics of the study and control groups were similar: the mothers' ages ranged from 18 to 39 years (mean, 26.8 ± 4.7 years in the control group and 27.1 ± 5.0 years in the study group), and parity was between 1 and 5 (1.7 ± 0.8) in the control group and 2.0 ± 0.8 in the study group). The weight gain of the mothers in both groups during pregnancy showed no significant difference (13.9 ± 4.5) in the control group, and 13.4 ± 4.3 in the study group). The educational level of the control group mothers was slightly higher than that of the study group (p < 0.019); the control group included fewer mothers with an elementary education and more mothers with university studies (Fig. 1). There was no difference in the fathers' educational level.

Onset of breastfeeding. On average, breastfeeding was initiated at 6.7 ± 8.0 hours postpartum in the control group and at 2.8 ± 2.3 hours in the study group. These findings were significantly different (p < 0.001).

Supplementary feeding of newborns in the mater-

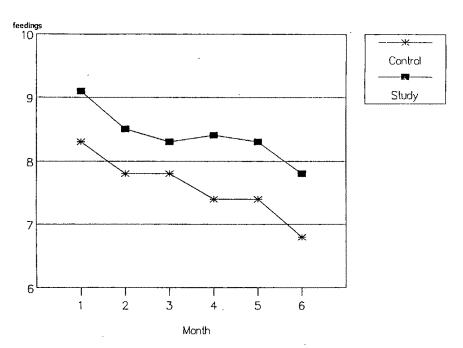


Fig. 2. Mean number of breastfeedings per day during the first 6 months postpartum.

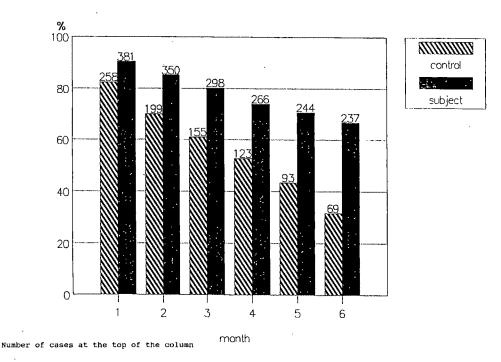


Fig. 3. Percentage of women exclusively breastfeeding and ammenorrheic during the first 6 months postpartum. (Illustration provided by Georgetown University.)

nity ward. Fifty-three percent of newborn infants from the control group received supplementary feeding during their stay in the maternity ward compared with only 19% of newborn infants in the study group (p < 0.001).

Breastfeeding frequency. Fig. 2 shows the mean number of breastfeeding episodes in 24 hours in the control and study groups during the first 6 months postpartum; the study group consistently had a higher number of feedings per day.

Status at the end of the sixth postpartum month. Of the control group mothers, 31.6% completed 180 postpartum days of exclusive breastfeeding compared with 66.8% of the study group mothers. At 180 days postpartum, 73 (23.3%) infants from the control group and

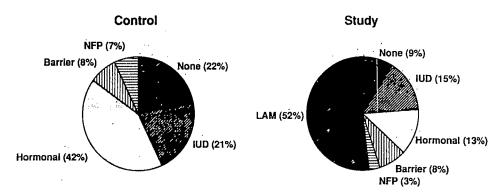


Fig. 4. Active family planning use at the end of 6 months postpartum.

Table I. Control and study group pregnancies during the first 6 months postpartum

Case No.	Postpartum mo pregnancy occurred	Vaginal bleeding	Exclusive breastfeeding.	Family planning
Control group				
156	4	Yes	No	No
235	4	Yes	Yes	No
74	5	No	No	NFP
119	6	Yes	No	No
187	6	Yes	No	Progestin
Study group				· ·
463	5	Yes	No	NFP
428	6	No	No	No
771	6	No	Yes	No
783	6	Yes	No	No

NFP, Natural family planning.

45 (10.7%) from the study group had been fully weaned from breastfeeding.

Forty-five percent of mothers from the control group and 64% from the study group remained amenorrheic for 180 postpartum days or more, whereas 69 (22.0%) mothers in the control group and 237 (56.2%) from the study group completed 180 postpartum days of exclusive breastfeeding and remained amenorrheic (Fig. 3).

Of the control group, 10.2% completed 180 postpartum days exclusively breastfeeding and amenorrheic, whereas 52.4% of the study group did so without the use of another form of family planning. The study group mothers had actively chosen to use amenorrhea as their method because of exclusive breastfeeding.

At the end of the sixth month, 66 (22%) mothers in the control group did not use any family planning method. Including LAM, the total active coverage increased from 78% in the control group to 91% in the study population. The selection of family planning methods in the control group was as follows: intrauterine devices, 21%; hormonal, 42%; barrier methods, 8%; natural family planning, 7%; and without family planning, 22%. It should be noted that 10% of those who were protected by the parameters of LAM did not

report it as a method. Method use among the study group was LAM, 52%; intrauterine devices, 15%; hormonal, 13%; barrier methods, 9%; natural family planning, 3%; and without contraception, 9% (Fig. 4).

Pregnancies. Five pregnancies were registered in the control group in the first 180 postpartum days, and four pregnancies were registered in the study group during the same period. The month that pregnancy occurred, the presence of menses, the state of breast-feeding, and the use of family planning when pregnancy occurred are presented in Table I.

Combining all mothers in both the control and study groups who completed 180 postpartum days in amenorrhea, who exclusively breastfed, who used no family planning methods other than LAM, and who were sexually active, only one pregnancy occurred. This yielded a 6-month cumulative life table pregnancy rate for LAM of 0.4% (Table II).

Growth curve (weight/age) of male and female infants exclusively breastfed for 6 months. Fig. 5 shows the growth curve of exclusively breastfed male and female infants in the study group during their first 6 months of life compared with the growth curve of the National Child Health studies. It may be seen that exclusively breastfed infants performed comparably.

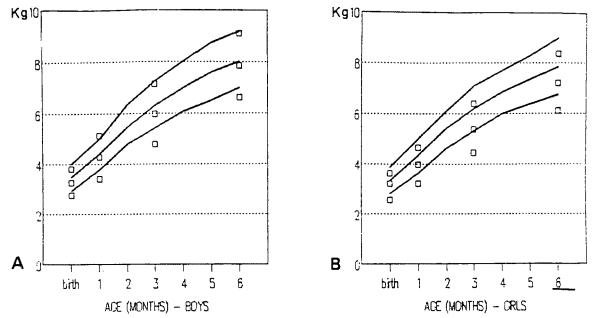


Fig. 5. Comparisons between National Child Health Studies growth curve (0) and weight/age of male (A) and female (B) infants in the study group exclusively breastfed during the first 6 months postpartum. (Illustration provided by Georgetown University.)

Table II. Life table on risk of pregnancy during the first 6 months postpartum in women exclusively breastfeeding and amenorrheic without contraception

Mo postpartum	Pregnancies	Women/mo	Accumulated women/mo	Rx 100	%
1	0	637	637	0.00	0.00
2	0	494	1131	0.00	0.00
3	0	380	1511	0.00	0.00
4	0	308	1819	0.00	0.00
5	0	269	2088	0.00	0.00
6	l	253	2341	0.40	0.39

Comment

The Breastfeeding Promotion Program for urban women of Santiago, Chile, appears to have had a positive impact on several paramaters related to breastfeeding. Changes occurred not only in the behavior of the study population but also among the health team in charge of the mothers and infants, as well as in the policies of the Departments of Obstetrics and Pediatrics. This statement is supported by the fact that in the study group, the period between delivery and first suckling was significantly reduced. Thus the average time postpartum to the initiation of breastfeeding in the study group was 2.8 hours, whereas 6.7 hours elapsed after delivery in the control group. Another piece of supporting evidence is that the number of newborn infants who received supplementary feeding during their stay in the maternity ward was significantly re-

Perhaps the most encouraging result of the Breastfeeding Promotion Program was the significant increase in the number of mothers in the study group who completed the sixth month postpartum exclusively or fully breastfeeding compared with control group mothers; only about 32% of the latter group was exclusively breastfeeding at 180 days postpartum, whereas 67% of the study group was exclusively breastfeeding at that time. This trend is reinforced by the data on completed weaning; at 6 months postpartum, 23% of the control group mothers had already weaned their baby, whereas only 10% of the mothers in the study group had weaned before 180 days postpartum.

In light of the Innocenti Declaration, which led to the statement accepted by the World Summit on Children, this breastfeeding promotion program contributed substantially toward optimal breastfeeding in this population. When analyzing the growth curves of the infants during the first 6 months of life, it can be seen that infants in the study group, who were exclusively breastfed during the first 6 months, showed a growth curve (50th percentile) that was actually higher than

that for international standards derived from the National Child Health Studies of the United States especially in female infants.

The number who completed 180 postpartum days in amenorrhea was also significantly higher in the study group (64.5%) compared with the control group (45%), as was the number who completed 180 postpartum days both amenorrheic and exclusively breastfeeding—56% in the study group and only 22% in the control group. This result reinforces the possibility that a program that encourages and promotes breastfeeding may have a profound impact on the duration of the infertile period. The intervention more than doubled the percentage of women who were naturally protected against an unplanned pregnancy, thereby reconfirming that full breastfeeding amenorrhea confers more than 98% protection against pregnancy during the first 6 months postpartum.15

The decrease in the use of alternative family planning methods during the first 6 months postpartum in the study group was more than overcome by the percentage accepting the natural breastfeeding/amenorrhea approach. LAM is acceptable, and breastfeeding promotion can play an important role in cost savings both in the hospital and by saving family planning resources within health services. 18 The 99.6% pregnancy protection rate demonstrated in this intervention for women in exclusive breastfeeding amenorrhea without family planning during the first 6 months postpartum corroborates the previous work in this area and the Bellagio Consensus. 15, 16, 19-22

In conclusion, breastfeeding promotion programs can have a positive impact on hospital policies and the health of women and children. If these programs include the fertility aspects (LAM), they can also contribute to child-spacing coverage, presenting an additional highly efficacious and natural option for women to consider.

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Discussion and recommendations

Discussion included the advantages of the incorporation of the lactation amenorrhea method (LAM) into natural family planning (NFP):

- It is simpler for couples newly interested in NFP.
 While they are using LAM, they have a chance to
 learn NFP in a more relaxed fashion than if they
 would have to apply all aspects of NFP at once.
- 2. It would delay the moment when it is necessary to start observing and charting the signs of NFP until the baby is older and the mother is less tired.
- 3. It would prevent the couple from observing unnecessary abstinence because of slight changes in fertility signs during the early postpartum months when in fact those changes have little significance.
- It is believed that the acceptability of NFP during breastfeeding would be increased if it were preceded by LAM, for the reasons mentioned previously.

Recommendations included:

- Improved, standardized nomenclature, definitions, and educational messages are needed, particularly with regard to potential fertility (fecundity).
- 2. Several research priorities were noted:
 - What are the implications of the Bellagio recommendations for different groups and countries? After 6 months?
 - What is the "within woman" variation in the duration of LAM and its potential fertility impact (i.e., can the duration of LAM and the return of potential fertility after one pregnancy

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- be used to predict the times of these events in a successive pregnancy)?
- What are the correlations between indices of returning potential fertility? Can additional markers be identified?
- Can methods be devised to reduce the falsepositive and false-negative signals of existing methods?
- Can the physiology of LAM and breastfeeding through the first year be better understood?
- What are the patterns of feeding within full breastfeeding that have the greatest impact on fertility suppression?
- What is the influence of nutritional factors and other clinical symptoms on returning fertility and the detection of existing clinical and biochemical indices?
- What is the role of pediatricians and other health workers in counseling women?
- 3. Education may be necessary to address the concerns of NFP providers. Some providers are reluctant to accept the 2% risk of pregnancy stated in Bellagio Consensus criteria; primarily, they may not realize that early pregnancies among their breastfeeding clients occur because these women are not within LAM criteria (fully breastfeeding, <6 months postpartum, and amenorrheic). A multiphase approach was suggested, which included presentations of scientific background of LAM to medical committees, training of trainers, curriculum development, and refresher training for teachers. Supervisory follow-up to support new activities could follow.

SESSION IV. NATURAL FAMILY PLANNING USE-EFFECTIVENESS AND CONTINUATION

Robert T. Kambic, Chair Suzanne Parenteau-Carreau, Rapporteur

Natural family planning use-effectiveness and continuation

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Numerous studies have been conducted to assess the use-effectiveness of natural family planning. However, because of imprecise definitions of terms, these studies show noncomparable results. Special effort is required to study natural family planning use-effectiveness by the same criteria as other family planning methods. (AM J OBSTET GYNECOL 1991;165:2046-8.)

Key words: Natural family planning, use-effectiveness, pregnancy rates

Any method of fertility control, natural family planning (NFP) included, has two major purposes; at the personal level it enables couples to have the number of children they choose, and at the aggregate level it moderates population growth so that the community, the common good, will benefit. To help couples plan their families, service programs must be able to communicate accurate information on how and how well a method can be used. Civil and medical authorities need to have confidence that the family planning methods they recommend will help their populace achieve better health.

Thus science has a responsibility to measure effectiveness, continuation, and other family planning evaluation parameters as accurately and reliably as possible. This has been done in large part for medical contraception but is only beginning for NFP.

Studying NFP use-effectiveness

There is a 10- to 15-year time lag between NFP evaluation and evaluation of other family planning methods. Tietze¹ cites 10 studies on tubal sterilization, 36 studies on oral contraceptives, and other studies on the intrauterine device and barrier methods, virtually all published in the mid to late 1960s. Many of these were studies of thousands of women. Tietze also cites two basal body temperature studies published in 1967 and 1968 with 17,500 and 5000 cycles, respectively. In contrast, the first large trial of the symptothermal method was the Rice study, which was first reported in 1977 with 1022 women²; the first large trial of the ovulation method, the World Health Organization study, published initial results in 1981.³ This time lag benefits NFP

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to the extent that it adapts the refined methodologies of family planning evaluation for its own use. It has hindered NFP because a lack of knowledge about the efficacy has up to now relegated NFP to a subordinate role in family planning.

When adapting family planning evaluation methodology for NFP application, knowledge about data collection, results generalization, statistical approaches, and developed-developing country comparisons can be transferred directly to NFP. However, there are other issues that only NFP research can resolve, e.g., the study population in NFP has to be precisely defined. Does a client enter the study at registration, when she is taught, when she begins to chart, or after she has charted for 1, 2, or more months? Definition of the study population is especially important to determine early dropout rates. Another example is the study unit: is it the woman or the couple? The woman charts her symptoms but assuming monogamy, both partners abstain. When considering pregnancies occurring in NFP users, there are two approaches: the accepted practice is to define a pregnancy according to the stated family planning intention of the client before a pregnancy occurs. An alternative practice recommended by some is to classify pregnancies according to the intercourse record of the client during the fertile time of the cycle in which the pregnancy occurred. If intercourse during the fertile time is always indicative of a desire for pregnancies, then unplanned pregnancies are virtually eliminated.

In summarizing the 1986 Ottawa conference of the International Federation for Family Life promotion, Dr. Paul Gross, who paraphrased Dr. Ronald Gray said

Professor Gray emphasized the dangers of defining these terms in NFP services in a way that does not allow easy comparison with the rates derived from services using other family planning methods. Because NFP will be judged by

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Table I. A decade of NFP effectiveness studies ranked by pregnancy rate; life table rates are cumulative net pregnancies at 1 year or 13 cycles per 100 women. (For definitions of life table and Pearl rates, see Labbok et al., which immediately follows.)

Country	Reference	Yr	Method	Life table	Pearl index
Indonesia	7	1990	OM	2.5	
Liberia	8	1990	ST/OM	4.3	
Korea	9	1989	OM	7.0	
Nepal	10	1986	MMM	7.3	
Zambia	8	1990	ST/OM	8.9	
Indonesia	7	1990	MMM	10.3	
Kenya	11	1988	OM	10.5	
United States	12	1981	ST	11.2	
Philippines	3	1981	OM	12.8	
Korea	11	1988	OM	13.4	
Bangladesh	11	1988	OM	14.9	
Ireland	3	1981	OM	17.4	
India	3	1981	OM	17.5	
Colombia	13	1980	ST	19.1	
Colombia	13	1980	OM	22.2	
United States	12	1981	OM	22.4	
El Salvador	3	1981	OM	26.9	
New Zealand	3	1981	OM	27.9	
India	14	1991	MMM		2.0
Germany	15	1991	ST		2.3
United Kingdom	16	1991	ST		2.7
Italy	17	1986	ST		3.7
China	18	1990	OM		4.4

OM, Ovulation methods; MMM, modified mucus method; ST, symptothermal method.

such comparisons, those dangers outweigh the advantages and leave the NFP movement open to unnecessary criticism.⁴

Of special interest are the simplified methods. It is not that people cannot use the standard NFP methods, but the simplified methods are less technical, easier to learn, and in some cases are literally being developed "in the field" by independent users (users not associated with an NFP program). Sociologists know that new, helpful knowledge will spread throughout a community by diffusion, that is, neighbors telling neighbors. NFP simplified methods may be currently undergoing diffusion and are important to a substantial proportion of couples in countries such as Mauritius, the Philippines, and Indonesia.

Communicating results

The most precise scientific results in the world are not helpful unless we use them. As Carl Djerassi⁵ pointed out, a crisis exists in the field of contraceptive development that has provided an opportunity for a fresh look at NFP. To take advantage of this opportunity, the results of effectiveness studies in NFP should be provided to those who will make use of them.

Table I lists NFP life table and Pearl index effectiveness studies reported during the past decade. Because of the methodology problems in measuring use-effectiveness, the data are not strictly comparable. (For a detailed discussion of the problems of comparing life table rates among studies and methodology problems in family planning evaluation, see Trussell and Kost⁶).

However, an examination of the life table rates in Table I is informative in several ways. There is a factor of about 10 between the lowest reported pregnancy rate of 2.5 and the highest rate of 27.9. Both are ovulation method studies. The median is 13.1.

Of the life table studies with pregnancy rates higher than the median, most were completed early in the 1980s. Of the studies with pregnancy rates lower than the median, all but one were completed after 1988. This suggests that throughout the 1980s, NFP programs improved their service, which enabled clients to achieve better effectiveness. This trend should be monitored.

When the Pearl index studies are included in the meta-analysis, we see a diverse group of countries at different levels of development where NFP services are sufficiently established to undertake a study of effectiveness rates. These countries are favorable sites for continued research and program improvement.

The articles presented in this section represent examples both of effectiveness studies and factors that impact on NFP effectiveness and continuation. They discuss a variety of approaches to these issues, reveal past methodologic errors, and provide information on NFP efficacy.

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Efficacy studies in natural family planning: Issues and management implications illustrated with data from five studies

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Studies of method effectiveness must be carefully assessed for comparability of findings. Several parameters are identified that are important in the assurance of comparable results. This article discusses these issues with the use of data from previously published studies and emphasizes the management implications of use-effectiveness data. (AM J OBSTET GYNECOL 1991;165:2048-51.)

Key words: Natural family planning, use-effectiveness, ovulation method

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Many family planning programs, whether natural family planning (NFP) or others, attempt to gather data to assess the effectiveness of the method(s) they are using. This is a useful effort not only for increasing understanding of the potential impact of the program but also for helping in management decisions. However, these data are not always easily gathered, and the manner of data collection can profoundly affect the outcomes and their comparability. This article discusses issues in use-effectiveness analysis and the application

Table I. Study design and sample

_	N	Median age (yr)	Entry
Chile: prospective, post-	419	28	Mo 1
Bangladesh: retrospective	201	26	Mo 4
Kenya I: retrospective	151	30	Autonomous
Korea: retrospective	168	32	Mo 1
Kenya II: prospective, interval	512	30	Mo 1

of such analyses to management decisions with examples from five studies.¹⁻⁴

Issues in data analysis

Contraceptive effectiveness is measured both clinically, for a "theoretical efficacy," and in actual usage, for "use efficacy." Clinical trials prospectively measure the efficacy of a method, whereas in-use measurements may be based on population surveys. The failure rate or the unplanned pregnancy rate is expressed as the number of pregnancies per 100 women or womanyears of exposure. This may be calculated as a Pearl index whereby the number of unplanned pregnancies is divided by the number of months of exposure and multiplied by 1200. The failure rate may also be calculated by the cumulative life table technique, an actuarial approach that is most often used to evaluate the first 12 months of use. Life tables permit the assessment of method efficacy over time and statistical comparison, but the Pearl index has the advantage of being easier to calculate.

A major problem in measurement of use-effectiveness is the accurate recording of the number of unplanned pregnancies that occur. Pregnancy reporting may suffer both from reporting bias and from loss to follow-up. These can be called "numerator problems" in that they affect the numerator in the calculations.

"Denominator problems" in NFP efficacy calculations can be illustrated with the data that is presented in Table I. These problems result from self-selection into the method, bias of recall, different times of entry into the studies, and, possibly from unreported concurrent use of other methods. As shown in Table I, clinical trials may have considerable variability among these parameters, and this may create difficulty in comparison of findings. For example, the Chilean study included a large population of postpartum women who were using the ovulation method (OM) and recording data each day; study entry was within 1 month of method acceptance. Studies in Bangladesh, Kenya I, and Korea were what might be called "prospective-retrospective;" that is, the data were gathered prospectively from time of entry but only as part of regular records, which were not designed to assure complete follow-up, but analyzed retrospectively.

Table II. Cumulative life table unplanned pregnancy rates and standard errors per 100 women who used the method for spacing or limiting

	9 Mo (%)	12 Mo (%)
Chile		
All women	6.9(1.4)	10.7 (1.8)
No breastfeeding	14.2 (3.9)	16.8 (4.0)
Any breastfeeding	4.5 (1.7)	
Bangladesh	10.1 (2.1)	14.9 (2.6)
Kenya I	10.4 (2.8)	10.4 (2.8)
Korea	9.5(2.4)	13.4 (2.8)
Kenya II	•	
All women	14 (1.7)	18 (1.9)
No breastfeeding	13 (2.6)	15 (2.8)
Any breastfeeding	14 (2.3)	19 (2.9)

In addition, it may be seen that the median age of the participants varied greatly and that the number of months of method use that were already completed at the time when data gathering began varied considerably. For example, the group in Kenya I did not enter the study until the acceptors had become autonomous users of the OM, whereas the group in Kenya II, which was a prospective study, entered the study whenever the couple first came to learn NFP. Another important issue is the comparability of the populations in each study. Age, for example, is known to affect fertility, and the clients who self-selected themselves into these programs were of different median ages.

When the data problems described above (different study designs, time of entry, self-selection and self-reporting, and consistency of method use and follow-up) are taken into account, the differences in the results that are presented in Table II are not surprising. For example, in an examination of only the three studies that followed a similar protocol (Bangladesh, Kenya I, and Korea), the lowest unplanned pregnancy rate is recorded when entry into the study was late, after autonomy was achieved. The center with the youngest population showed the highest unplanned pregnancy rate. The studies in Chile and Kenya II allow us to look at the breastfeeding versus nonbreastfeeding populations. When study entry is immediately after delivery (Chile), breastfeeding is protective against unplanned pregnancy, but when study entry occurs at any time during the interbirth interval, breastfeeding apparently is associated with an increase in unplanned preg-

Unplanned pregnancy rates were calculated only for those who used the method to avoid conception. Among studies that are presented herein, unplanned pregnancy rates varied from 10% to 19% with 95% confidence intervals that encompass a range of 4.8% to 25% (Table II). Life table rates for studies in the United

Table III.	Categorization	of pregnancies	s reported as uni	planned regardles	s of entry intention

	Ch	vile	Bang	ladesh	Ken	ya I	· Ko	rea	Ken	ya II
	N	%	·N	. %	N ,	%	Ň	%	N	% .
Entered study	475		291		199		189		478	
Reported unplanned pregnancies	50	100	48	100 ·	14	100	21	100	88	100
Method-related	9	. 18					2	9	2	2
User-related	40	80	37	77	14	100	18	86	68	7
Rules not under- stood	3	6	11	23	4	29	17	81	25	28
Rules not under- stood but not fol- lowed	37	74	26	54	10	71	1	5	43	49
Unknown	1	2	11	23	0	0	1	5	18	21

States and other developed regions range from 16 to 27.5.6 Three prospective studies in developing countries and a World Health Organization (WHO) collaborative study found rates between 5 and 24.7 Early postpartum breastfeeders who use the OM seem to benefit from the fertility-reducing side effect of lactation, whereas the late breastfeeders seem to have more difficulty in using the OM to avoid pregnancy. It should also be noted that use-effectiveness for other forms of family planning in similar settings is not well known. It is not unreasonable to assume that these rates may be comparable to those of other methods under these conditions and in these settings.

At a meeting on cost-effectiveness, which was sponsored by the Institute for International Studies in Natural Family Planning in 1988, an alternative plan to address unplanned pregnancy rates was suggested. It was suggested that the percent of clients who achieve adequate spacing or who achieve their stated goal might be a better indicator of success. This approach to effectiveness assessment has three advantages: it is a personalized indicator that reflects the couples' plans, it may be easier to assess, and it would give a manager excellent information over time. The major disadvantage is that this approach disallows comparisons between methods and among programs, an activity that is often necessary for funding support and method promotion.

Use of efficacy data in management decisions

A better understanding of efficacy data and how it is gathered can lead to improved management decision making. Life tables can give us an understanding of many issues, especially when multiple-decrement life tables are used. In the Chilean study, 12-month life tables indicate that about 12% of the women dropped out to become pregnant, 12% experienced an unplanned pregnancy, and 7% changed to another method for a total discontinuation rate of about 32%. The remaining 68% would represent the total cumu-

lative continuation rate. By similar analysis the three countries (Bangladesh, Kenya I, and Korea) had approximately 67% continuation and Kenya II, in part because of the higher unplanned pregnancy rate, had a continuation rate of 50%. The continuation rate is an indicator of the percent of acceptors who are still using NFP at the end of 1 year. In assessment of a program, low continuation rates should alert the manager to consider program modification. By assessing the contribution of each element to the discontinuation (pregnancy, switching, drop-out), the manager can be alerted to the most appropriate intervention approach.

Another area of analysis that is helpful in management is an exploration of the unplanned pregnancies by category. Table III illustrates the distribution of the unplanned pregnancies in these five studies by several categories. This first breakdown is by method- and user-related categories. In user-related categories, two options were considered: (1) when the rules are not understood (whether this is due to problems with the teacher or the student) and (2) when the rules are understood but are not following the cycle of conception. In addition, there is an unknown or undetermined category.

An example of how examination of these categories may help a manager is seen in the study in Chile. This is the study with the highest percentage of breastfeeders; there is also a higher method-related failure rate. The manager who is seeking an explanation would find that the majority of these user-related pregnancies occurred during lactation but after return of menses and therefore after return of fertility. The manager could thus address program resources to this issue.⁹

The most common contributor to the user-related category is when knowledgeable users do not follow the rules of the ovulation method. However, in Korea many of the failures were attributed to misunderstanding of the rules as a result of teaching problems. The high number of unplanned pregnancies that were categorized as unknowns in the Korea and Bangladesh studies

may be the result of cultural hesitance to record the occurrence of sexual relations. This information allows the manager of the program to reassess teaching methods, learner knowledge, and behavioral difficulties. An intervention may target teacher's education or client support to assist in understanding or following the rules of NFP.

Studies of effectiveness allow program managers to judge their program in comparison with studies that are reported in the literature. Comparisons can be made in terms of those variables that are frequently associated with unplanned pregnancy (e.g., maternal youth), and progress can be monitored within that category over time. Data in the five studies that are discussed in this article seem to show that some variables that have been generally accepted as associated with unplanned pregnancy lose that association when other variables are properly controlled.^{1, 3}

For example, we find that when lactation status is adequately controlled, the difference in pregnancy rates between spacers and limiters virtually disappears. It should be noted, however, that the clients' stated receptivity to an unplanned pregnancy is associated with the occurrence of unplanned pregnancy; this stated receptivity occurred both among spacers and limiters. Therefore it is possible that this is a much better indicator of the need for special counseling than is a statement about spacing versus limiting.

Conclusions

It is clear that all of the above information has implications for management. Therefore the study of efficacy is not simply for academic purposes but to improve management. Those who develop management information systems should consider including measures of NFP efficacy that will allow the program manager to make adjustments that are necessary to improve the impact of the program.

The data that were used to illustrate ovulation method use-effectiveness issues are from studies coauthored with Dr. Alfredo Perez, Dr. Hanna Klaus, Ms. Teresa Muruthi, Mrs. Farida Shah, and Dr. Mark Jacobson. We thank Dianne Barker and Ms. Rebecca Stallings for research assistance.

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Effectiveness and acceptability of the symptothermal method of natural family planning in Germany

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Throughout Germany, 851 women who were instructed in natural family planning participated in a prospective study. Of these, 255 women with 3174 cycles used only natural family planning for family planning and 274 women with 3995 cycles occasionally used barrier methods in the fertile phase. For natural family planning—only users, the Pearl rate for unplanned pregnancy was 2.3 and for mixed-method users 2.1. Most pregnancies resulted from unprotected intercourse during the fertile phase, and the use of barrier methods does not reduce risk-taking. (AM J OBSTET GYNECOL 1991;165:2052-4.)

Key words: Natural family planning, effectiveness, symptothermal method

In 1985, a representative public opinion poll showed that in Germany about 4% of the women between 15 and 45 years of age use a natural method of family planning (NFP). The main reason German couples adopt NFP is dissatisfaction with other contraceptive methods or fear of their side effects.

In a prospective study supported by the German Ministry for Youth, Family, Women and Health,¹ we have studied the use-effectiveness and the acceptability of the symptothermal method of NFP, the sexual behavior of the users, and the proportion of women or couples who use NFP only ("NFP users") or who use barrier methods in the fertile phase ("mixed-methods users"). Furthermore, we also wanted to know how many couples avoiding pregnancy have intercourse in the fertile phase without any contraceptive protection. The study, begun in 1984, is in progress and is a joint project of the University of Düsseldorf and the Arbeitsgruppe NFP, Bonn.

Material and methods

The study participants and, when possible, their partners have been instructed in small groups by about 500 NFP teachers. They were instructed in the symptothermal method, (STM) which mainly uses self-observation of the cervical mucus and basal body temperature to define the beginning and the end of the fertile phase of the cycle.² To avoid pregnancy, no intercourse should take place in the fertile phase.

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Table I. Sexual behavior in fertile phase (N = 506)

	%
NFP only	
No intercourse in fertile phase	12.5
Occasional unprotected intercourse in fertile	34.2
phase	
Mixed method	
Occasional protected intercourse	11.3
Occasional protected and unprotected intercourse	42.1

The woman's observations are entered on a specially developed cycle chart. The woman also records sexual behavior including protected and unprotected intercourse and other forms of genital contact. In addition, the client states on each chart whether or not she wants to become pregnant in the next cycle. Pregnancies are classified as "intended" or "unintended" on the basis of the statements made before conception. To enter the effectiveness study, the participants must be between 20 and 45 years of age and have a cycle length of 25 to 35 days.

All women who request NFP are taught, including women with irregular cycles, women who have discontinued oral contraceptives, and women who are premenopausal, are breastfeeding, have had an abortion, and are trying to conceive. Within the prospective study, a woman may first use NFP in the postpartum situation, then may be in the use-effectiveness study for some cycles, and then try to achieve a pregnancy.

A self-developed computer program serves for data collection, statistical evaluation, supervision and organization of the NFP teachers, and administration. With use of this program, interim results can be reviewed at any time.

Table II. Significant differences between consistent and inconsistent users

	Consistent users $(n = 120)$ (%)	Inconsistent users (n = 386) (%)
Working or training/housewife	69/31	53/47*
Unmarried/married	44/56	26/74*
No child/one or more children	62/38	46/54*

There were no significant differences in age, educational level, completed family size (spacer/limiter), unintended pregnancy before study entry, experience with other family planning methods, or religion. *p < 0.05.

Results

Altogether there are 851 women with 12,765 cycles in the study. Of these, 506 women with a total of 6891 cycles met the conditions of the effectiveness study. Of these, 241 women with 3007 cycles used only NFP for family planning and 265 women with 3884 cycles occasionally used barrier methods, mainly the condom, in the fertile phase.

Women with a cycle length of <25 or >35 days are often excluded from effectiveness studies because of the assumption that they have reduced fertility. However, we found that women with short cycles have fully adequate cycles with early ovulations and sufficiently long luteal phases (high-temperature phase of 10 days or longer). Only cycle lengths of <22 days are often connected with signs of subfertility. The high fertility of women with short cycles has been shown by the relatively high number of unintended pregnancies among them, and we therefore included them in the use-effectiveness study. Therefore, for the NFP-only users there were two more unintended pregnancies in 167 cycles and for mixed-method users one more unintended pregnancy in 111 cycles. The Pearl Index for NFP-only users (with a cycle length of 22 to 35 days) was 2.3 and for mixed-methods users was 2.1.

The educational level of NFP clients is higher than in the general population. Fifty-nine percent have attended grammar school or the university. One reason for this circumstance is that information on NFP is obtained through books or by means of special educational institutions; little information can be obtained via the media or physicians. The participants are comparatively young, with 67% between 19 and 29 years old, and almost half of them are childless. Seventy-three percent of the women want more children in the future and are spacing. Eighteen percent have completed their family size and are limiting. There are no significant differences between NFP-only users and mixed-methods users. The lost-to-follow-up rate is 2.6%, and dropouts because of dissatisfaction and problems with NFP are 7.1%.

In the use-effectiveness study for NFP-only users, we found four unintended pregnancies in 3007 cycles, which results in a Pearl rate of 1.6. For mixed-methods users, the Pearl Index was 1.9 with six unintended pregnancies in 3884 cycles.

Of the 13 unintended pregnancies, three were method failures. The other 10 pregnancies occurred in cycles in which unprotected intercourse took place during the fertile phase. This means that those who at times used barrier methods also had unprotected intercourse during the fertile phase. Many user-related pregnancies occur because couples, though refraining from intercourse in the highly fertile phase, risk unprotected intercourse just at the beginning of the fertile phase believing "nothing will happen this time."

Among NFP-only users there were two method failures. In both cases the coitus responsible for the conception took place on the fifth cycle day, once 7 days before temperature rise and in the other case 5 days before temperature rise. The third method failure occurred in the mixed-methods group, when there was no intercourse in the fertile phase.

Table I shows how couples behaved in the fertile phase. Groups 1 and 3 were the consistent users whereas groups 2 and 4 were the risk-taking groups that occasionally had unprotected intercourse during the fertile phase. It is surprising that those who sometimes used barrier methods still had unprotected intercourse in the fertile phase. Thus, the use of barrier methods does not reduce additional risk-taking.

Table II compares the consistent users (groups 1 and 3 of Table I) with the risk-taking group (groups 2 and 4 of Table I). This table shows that women who were working or in training outside the home, women who were unmarried, and women with no children were more likely to be consistent users (p < 0.05). Conversely, women who were housewives, were married, or had children were more likely to take risks (p < 0.05). Consistent users may have been more motivated to avoid pregnancy.

Comment

Many prospective studies deal with effectiveness of NFP in Europe, in the United States, and in a number of developing countries.3-9 Our study adds information about sexual behavior in the fertile phase of the cycle. In the current study, the unplanned pregnancy

rate was low and was comparable to that with use of the intrauterine contraceptive device. The other studies show unplanned pregnancy rates that differ widely from each other and range from very good to very poor because of methodologic or cultural factors. We found that Germany NFP users frequently took risks and had unprotected intercourse in the fertile phase. They mostly broke the rules at the beginning of the fertile phase when the risk of pregnancy was rather low.

Summarizing, the use-effectiveness of the examined symptothermal method is very good. It is true that users do not always behave consistently. If, however, they have unprotected intercourse during the fertile phase, they do so at the beginning of the fertile phase knowing they are taking a risk. The low drop-out rate shows good acceptance. About half of the women or couples who are taught sometimes use barrier methods in the fertile phase.

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Further analysis of contraceptive failure of the ovulation method

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Reanalysis of data on the ovulation method of natural family planning collected by the World Health Organization yields the following conclusions. The method is effective during perfect (correct and consistent) use, with a first-year probability of failure of 3.4%. However, it is extremely unforgiving of imperfect use, with a first-year probability of failure of 84.2% if the method is not used correctly. During the initial year, 87% of the cycles were characterized by perfect use. Nevertheless, the 13% of cycles characterized by imperfect use had a tremendous impact on the overall failure rate. During the first year of typical use, 22.5% of the women in the clinical trial became accidentally pregnant. (AM J OBSTET GYNECOL 1991;165:2054-9.)

Key words: Natural family planning, ovulation method, use-effectiveness

The standard procedure for calculating separate failure rates caused by method and user error is logically flawed and produces estimates that are biased down-

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ward, with the extent of bias unknown. In a previous paper, we provided correctly calculated estimates of method and user efficacy for the ovulation method (OM). Using data collected in a clinical trial conducted by The World Health Organization (WHO), We analyzed failure rates separately for perfect use (when the method is used correctly and consistently) and imperfect use. Probabilities of a contraceptive failure during the first year after successful completion of a teaching phase are 3.1% during perfect use and 86.4% during imperfect use. We concluded that if used perfectly, OM is very effective in preventing pregnancy. However, it

is extremely unforgiving of imperfect use, especially breaking rules forbidding intercourse during the fertile period or during times of stress. Breaking these most serious OM rules entails a 27% risk of pregnancy per cycle.

Our previous analysis of OM efficacy was based on the 13-cycle effectiveness phase of the WHO study. The effectiveness phase was preceded by a teaching phase that lasted from three to six cycles. The purpose of the present study is to determine whether analysis of efficacy during the teaching phase can enhance understanding of the probabilities of failure during perfect and imperfect use and of the pregnancy risks of particular forms of imperfect use. We also combine the data from the teaching and effectiveness phases to compute the probability of failure during the actual first year of use of OM so that the result will be comparable with the results for other methods without a separate teaching phase. Finally, we explore further the relationship between the probabilities of failure during perfect and imperfect use and the probability of failure during typical use and the meaning of this formal relationship for counseling clients.

Data

WHO invited five centers with experience in teaching OM to participate in a clinical trial. The centers were located in Auckland (New Zealand), Bangalore (India), Dublin (Ireland), Manila (Philippines), and San Miguel (El Salvador). Participants in the trial were required to be less than 39 years old, not to be lactating, to have a history of menstrual cycles of between 23 and 35 days, to have had at least 1 live birth (or term stillbirth) within the preceding 5 years in the present union, to be cohabiting, not to have used hormonal contraception for at least three cycles before admission, not to have used OM previously, to agree not to use any other method of contraception during the effectiveness phase of the study, and to be an informed volunteer willing to keep the necessary records.

A total of 869 women were admitted to the teaching phase of three to six cycles. Of these, 765 (88%) successfully completed the teaching phase, and 725 entered the effectiveness phase (with the remaining 40 patients withdrawing for a variety of reasons, including desire for pregnancy, dissatisfaction with the method, and moving away). The OM rules, which are described in greater detail in Table I of our previous paper, forbid intercourse during menses, on alternative early "dry" days before the start of the fertile period (to minimize confusion between seminal fluid and cervical mucus), during the fertile period itself, and during periods of stress. The fertile period begins with the onset of mucus secretion, peaks on the last day on which "fertile" mucus (resembling raw egg-white) is recog-

nized, and ends on the evening of the fourth day past the peak day. Intercourse is forbidden on roughly half the days in each cycle. The first cycle of the teaching phase had a completely different rule—intercourse was forbidden altogether—with important implications for the analysis of efficacy of OM (discussed below).

Separate data collection forms were used for each menstrual cycle, with each subject recording fertile days, days of bleeding, and days of intercourse. From the chart and a monthly meeting during which the teacher carefully questioned the subject, the teacher transcribed selected cycle details onto a paper form and judged compliance, comprehension, and attitude. Totals of 2701 cycles were recorded for the 869 women who entered the teaching phase and 7514 cycles for the 725 women who entered the effectivness phase. Copies of all forms were checked for consistency and completeness at WHO headquarters, where an analysis file in machine-readable format was created. This analysis file was generously provided to us by Dr. P. F. A. Van Look at WHO.

The forms for the teaching and effectiveness phases differ in several important ways that hamper analysis of the combined data. First, as previously mentioned, the initial cycle in the teaching phase had an additional rule that forbade intercourse. Second, there were several additional categories of imperfect use on the teaching phase forms that did not exist on the effectiveness phase forms, including improper charting, intercourse on the peak day, and intercourse around the time of a second peak. Categories common to forms in both phases were intercourse during times of stress, intercourse on consecutive dry days, intercourse on mucus/wet days, intercourse within 3 days after peak, genital contact on wet days, intercourse during bleeding/spotting, and use of other methods. Thus it is not clear how uniquely to map categories during the teaching phase into categories during the effectiveness phase. Of particular concern is the fact that improper charting usually precludes even knowing which real rules were broken.

Finally, the form for the teaching phase recorded only whether or not coitus occurred during the cycle and not how many times; the form for the effectiveness phase recorded total acts of intercourse, but it is clear that the number of acts was underreported. Women were asked to report all acts of intercourse and required to record the last act before the onset of the fecund period, all acts during the fecund period, the first act after the end of the fecund period, and whether all acts during the cycle were reported. It is clear that not all acts of intercourse during the fecund period were reported. Conception rates per cycle day are much higher in the WHO trial than in other studies, and the WHO investigators themselves concluded that under

reporting of intercourse occurred during the fecund phase.⁵ Furthermore, frequency of intercourse (even among those who reported that they recorded all acts) was unrelated to the probability of failure during perfect use and imperfect use. Unless the risk per exposure of an event occurring is zero, then it logically follows that the more times one is exposed, the greater should be the probability that the event will occur.

In our analyses, we discarded 560 cycles in the teaching phase and 30 cycles in the effectiveness phase during which women reported that no intercourse had occurred. We retained 46 cycles in the teaching phase and 156 cycles in the effectiveness phase for which insufficient information was available to determine whether intercourse had occurred; in short, we assumed intercourse occurred unless there was direct evidence to the contrary. One might object that those who do not have intercourse in a cycle are nevertheless perfect users of OM. However, women simply are not exposed to the risk of contraceptive failure unless they have intercourse, so that periods characterized by no exposure to risk of pregnancy should be removed. 8-10

Moreover, all qualitative results would be identical regardless of the inclusion or exclusion of these cases, and the quantitative impact of including the cycles with insufficient information to determine whether coitus had occurred or excluding the cycles during which intercourse did not occur is trivial. The total number of cycles included in the analyses is 2141 in the teaching phase and 7484 in the effectiveness phase. Finally, we were forced to impute perfect/imperfect use in a small number of cases in which data were missing; only for one cycle in which pregnancy occurred (in the effectiveness phase) did we have to impute perfect/imperfect use.

Results

How to treat the first cycle is a vexing issue, because intercourse was forbidden in that cycle. Nevertheless, 397 of 869 couples (46%) definitely disobeyed this rule, eight of whom (2.0%) became pregnant. In 16 cases there is no evidence whatsoever whether the couples had intercourse, and in two cases whether the couples had intercourse was left blank, but additional information indicated that no rules were broken. In subsequent analyses involving the initial cycle, these 16 cases were coded as having intercourse (and thus disobeying the rules in cycle 1), and the two cases were coded as not having intercourse (and thus obeying the rules) to be consistent with our general imputation procedure that we eliminated cycles only if there was evidence that no coitus occurred.

By definition, those who used OM perfectly during the first cycle did not have intercourse and thus were not exposed to the risk of pregnancy. As stated above, cycles in which couples are not exposed to risk should be removed from the analysis of contraceptive efficacy. Nevertheless, if we count all 869 cycles of exposure in the first cycle of the teaching phase (regardless of whether intercourse occurred), then the life table probability of failure in the initial year (cycles 1 to 13) of use is 22.0%. If we skip the first cycle altogether, the first-year (cycles 2 to 14) probability of failure is 21.9%. If we count only those initial cycles in which intercourse occurred, the initial-year probability of failure rises to 22.8%.

Life table probabilities of failure during the first year (cycles I to 13) are 84.1% during imperfect use and 3.2% during perfect use; if the initial teaching cycle is excluded, these probabilities rise slightly, to 85.0% and 3.4%, respectively. Hence the life table probability of failure in the initial year of use is fairly insensitive to the treatment of the initial cycle of the teaching phase. For this reason and because rules are so fundamentally different, we discard this initial cycle in all subsequent analyses.

During cycles 2 to 6 of the teaching phase, there were 37 pregnancies in the 1728 cycles in which intercourse occurred, or a 2.1% failure rate per cycle. Three pregnancies occurred during 1386 cycles of perfect use (failure rate = 0.22%) and 34 occurred pregnancies during 342 cycles of imperfect use (failure rate = 9.9%). The failure rate per cycle during perfect use (0.22%) was nearly identical to and not statistically different from the failure rate during perfect use in the effectiveness phase (0.24%). This result conforms to the hypothesis that we articulated in our previous paper, because we reasoned that during perfect use, only biologic fecundity and frequency of intercourse should affect the propensity to fail and that these factors would not differ much between the teaching and effectiveness phases.

In contrast, the failure rate per cycle during imperfect use (9.9%) was significantly lower than the failure rate during imperfect use in the effectiveness phase (14.1%). Two factors could account for this difference: (1) On average the rules couples break in the teaching phase might be expected to be less serious than the rules broken in the effectiveness phase, and (2) on average the number of times couples break rules per imperfect-use cycle might be expected to be lower in the teaching than in the effectiveness phase. We expect that both factors would operate because couples are likely to be more careful until they gain experience with OM; both could result from less frequent intercourse during the teaching phase. Couples break rules more often in the teaching phase—20% of cycles were characterized by imperfect use compared with only 11% in the effectiveness phase-probably because of an imperfect understanding of OM during the initial cycles of the teaching phase.

Table I. Pearl index failure rates by type of rule-breaking during the teaching phase

	Pregnancies	Cycles	% per cycle	% per yr*	Confidence interval†
Total ·	37	1728	2.1	24.5	17.4-31.3‡
Obeyed rules	. 3	1386	0.2	2.8	1.0-4.5‡
Disobeyed rules	34	342	9.9	74.4	59.8-83.9‡
Broke this rule and perhaps others			•		
Consecutive early days	1	72	1.4	16.6	0.5-63.7
Mucus/wet days	10	54	18.5	93.0	71.7-99.3
3 Days after peak	17	63	27.0	98.3	90.5-99.9
Genital contact in wet days	1	9	11.1	78.4	3.6-100.0
Bleeding/spotting	4	67	6.0	55.1	19.5-87.1
Situations of stress	1 .	2	50.0	100.0	15.2-100.0
Peak day	8	12	66.7	100.0	99.6-100.0
Around second peak	2 5	5	40.0	99.9	50.6-100.0
Mischarting	5 .	59	8.5	68.4	31.0-93.2
Broke only this rule					
Consecutive early days only	0 '	64	0.0	0.0	0.0 - 4.6
Mucus/wet days only	. 1	31	3.2	34.7	1.1-90.7
3 Days after peak only	. 11	47	23.4	96.9	81.9-99.8
Genital contact in wet days only	1	4	25.0	97.6	7.9-100.0
Bleeding/spotting only	0	46	0.0	0.0	0.0 - 6.3
Situations of stress only	0	-0			
Peak day	3 .	3 .	100.0	100.0	36.8-100.0
Around second peak	1	2	50.0	100.0	15.2-100.0
Mischarting	2	51	3.9	40.6	6.0-84.7

^{*}Calculated as $(1 - [1 - P^{18}]$, where P is the probability per cycle (number of pregnancies per number of cycles). Note that this annual probability is purely notional, since the teaching phase lasted 6 months at most.

Probabilities of failure per cycle and per year are shown in Table I for overall use, perfect use, imperfect use, and specific types of imperfect use during the teaching phase. The probabilities presented in Table I are based on Pearl index and not life table calculations. For these data, differences in cumulative failure rates from the two methods are nearly identical, because duration-specific rates do not vary much. Specific types of imperfect use are shown in two panels. The upper panel pertains to cycles in which a specific rule was broken regardless of whether other rules were also broken; the lower panel pertains to those cycles in which the specific rule listed was the only rule broken.

The upper panel of Table I indicates that having intercourse on mucus/wet days, within 3 days after the peak, during situations of stress, on the peak day, or around a second peak are particularly risky, with an implied annual probability of failure exceeding 90%. Having intercourse during cycles that were mischarted or during bleeding/spotting or having genital contract during wet days entails a high risk, with an annual probability of failure exceeding 50%. Having intercourse on consecutive early days has the lowest risk (16.6% would fail in the first year). However, it is possible that breaking a specific rule only appears to be risky because it is broken in combination with other rules that do entail great risk when broken. The only way to

answer this question is to examine cycles in which only one rule was broken.

The lower panel of Table I confirms that having intercourse within 3 days after the peak, on the peak day, or around a second peak is indeed risky; breaking any of these rules entails at least a 95% chance of pregnancy within a year. However, the risk of pregnancy from intercourse on mucus/wet days, during bleeding/spotting, or during cycles that were mischarted is substantially lower than would be indicated by the upper panel; when these rules are broken (in the upper panel), more serious rules seem to be broken as well.

It is difficult to compare the risks entailed by breaking specific rules in the teaching phase with the corresponding risks in the effectiveness phase because imperfect use was coded differently in the two phases. Specifically, the last three categories shown in Table I do not exist for the effectiveness phase. Our previous analysis of the effectiveness phase indicated that rules with the most serious consequences when broken were those forbidding intercourse on mucus/wet days, within 3 days after the peak, and during situations of stress. Breaking the rules forbidding intercourse during bleeding/spotting and genital contact on wet days entailed the smallest risk. The risk from intercourse on consecutive early days was intermediate.

The results in Table I are in general agreement with

[†]Annual 95% confidence interval computed from cycle confidence interval based on exact binomial. Cycle confidence interval has 2.5% area in each tail except when p = 0.0 or p = 1.0, in which cases the cycle confidence interval has 5% area in one tail.

[‡]Annual 95% confidence interval computed from cycle confidence interval based on normal approximation. Cycle confidence interval has 2.5% area in each tail.

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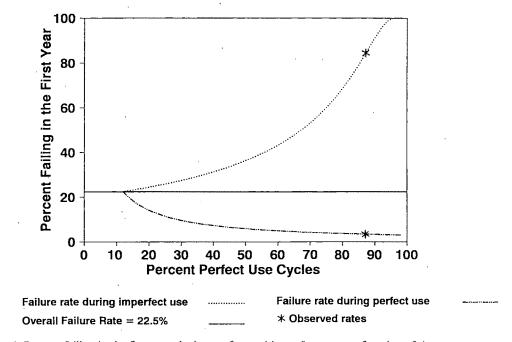


Fig. 1. Percent failing in the first year during perfect and imperfect use as a function of the extent of perfect use.

our prior findings with the following two exceptions: (1) intercourse on consecutive early days has a lower risk in the teaching phase, and (2) intercourse on mucus/wet days has a far lower risk in the teaching phase. The risk of pregnancy from intercourse on mucus/wet days in the teaching phase, when that is the only rule broken, seems implausibly low. Because this particular risk is so low, because we cannot match the imperfect use categories for the two phases, and because we suspect that specific types of imperfect use would be more precisely identified in the effectiveness phase when the couples had acquired better charting skills, we conclude that our prior results for the effectiveness phase more accurately reflect the true risks of breaking particular rules.

Implications of misreporting

Classification of exposure cycles as perfect or imperfect clearly requires accurate reporting of behaviors. It is plausible that imperfect use would be underreported, given the understandable human tendency not to emphasize one's failings. Underreporting seems far less likely in those cycles in which pregnancy did occur, because the investigators examined the circumstances surrounding all pregnancies especially carefully. If so, then our perfect-use failure rates are too low, but our imperfect-use rates are too high. In this section we explore more formally the consequences of misreporting.

In the first year of use (cycles 2 to 14) of OM during

the WHO trial, 159 pregnancies occurred in 8209 cycles. This 1.94% failure rate per cycle translates into 22.5% of couples failing in the first year of use. There were 19 pregnancies in 7150 perfect-use cycles (3.4% of couples failing in the first year) and 140 pregnancies in 1059 imperfect-use cycles (84.2% failing in the first year). The typical per cycle failure rate is the weighted average of the per cycle failure rates for perfect and imperfect use: $f_i = \alpha f_p + (1 - \alpha) f_i$, where f_i , f_p , and f_i are per cycle failure rates for typical, perfect, and imperfect use, respectively, and α is the proportion of perfect-use cycles. Note that α can be interpreted as an index of how easy the method is to use correctly and consistently; the smaller is α , the more difficult it is to use the method perfectly. We regard these four pieces of information (f_i , f_p , f_i , and α), only three of which are independent, as necessary for a couple to make a truly informed choice of a contraceptive method.

If we assume that pregnancies are properly classified as occurring during perfect or imperfect use, although perfect or imperfect use in cycles in which pregnancy did not occur might be misreported, then we allow for the possibility that the failure rates that we have computed for perfect and imperfect use are incorrect. Nevertheless, the following equation holds for the OM data: $f_1 = 0.0194 = (n/8209) (19/n) + ([8209 - n]/8209) (140/[8209 - n])$, where n is the true number of perfect use cycles, 19/n is the true failure rate during perfect use, and 140/(8209 - n) is the true failure rate

during imperfect use. We are led to the inescapable fact that if the failure rate during imperfect use is lower than the rate reported, then the failure rate during perfect use must be higher than that reported.

Fig. 1 shows the annual probabilities of failure implied by changing n from its reported value of 7150 ($\alpha = 0.871$) to a low of 981 ($\alpha = 0.119$) that would result in $f_p = f_t$ to a high of 8069 ($\alpha = 0.983$) that would result if all cycles were perfect-use cycles except the 140 in which pregnancy occurred during imperfect use. In constructing this figure, we assume that the failure rate during perfect use cannot exceed the failure rate during imperfect use. These results imply that at one extreme OM is very forgiving of imperfect use but has very low efficacy in preventing pregnancy and is very difficult to use perfectly.

At the other extreme, OM has high efficacy in preventing pregnancy if used perfectly but is extremely unforgiving of imperfect use and very easy to use perfectly. Our previous conclusion, based on the reports of perfect and imperfect use in the data, falls near the latter extreme. We have concluded that OM is extremely unforgiving of imperfect use and moderately difficult to use perfectly ($\alpha = 0.871$, thus OM is used imperfectly in 13% of cycles). If one wished to argue that the method is in fact more forgiving, because our estimate of imperfect-use efficacy is biased upward by misreporting of rule-breaking, then it must also be true that the method when used perfectly has lower efficacy and that OM is more difficult to use perfectly.

In conclusion rule-breaking during the teaching phase of the OM trial was more common than rulebreaking during the effectiveness phase. One would expect this finding given that understanding of the method and charting skills improve with experience. Fortunately, it seems that the specific rules broken in the teaching phase seem to have consequences on average that are less serious than rules broken in the effectiveness phase. Two findings seem particularly relevant to counseling. First, the rule forbidding intercourse during the first teaching cycle was disregarded by nearly half the couples in the trial, among whom 2% became pregnant. Given this fact, it would seem advisable to emphasize during the initial teaching session that couples not have intercourse before charting skills are established. Further, couples should be counseled to use an adjunct (nonhormonal) method if they cannot adhere to this rule. Second, couples should be advised that OM is quite effective at preventing pregnancy when used perfectly but that it is extremely unforgiving of imperfect use.

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Factors related to autonomy and discontinuation of use of natural family planning for women in Liberia and Zambia

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From 1983 to 1988, natural family planning programs were conducted in Liberia and Zambia. In Liberia 1055 and in Zambia 2709 women used natural family planning to avoid pregnancy. These users could become pregnant, discontinue use of the method, or become autonomous users. Women who changed intention did not stop use of natural family planning and were not treated as discontinuations. In a multivariate analysis, client's age, breastfeeding status, employment, urban/rural residence, time of registration in the program, and visit intensity were significantly associated with the outcomes. The most consistent association was that women who entered the programs in the later time periods were more likely to become autonomous users and less likely to discontinue use of the method or to experience an accidental pregnancy. (AM J OBSTET GYNECOL 1991;165:2060-2.)

Key words: Natural family planning, autonomous users, unplanned pregnancy. Liberia, Zambia

From 1983 to 1988, natural family planning (NFP) demonstration programs were conducted in Liberia and Zambia. The overall evaluation of these programs has been reported in a companion volume to this publication. Here the focus is on factors that are associated with autonomous use, discontinuation of use, and unplanned pregnancy among women who wanted to avoid pregnancy in Liberia (1055) and in Zambia (2709).

Methods

At registration, data were collected on socioeconomic characteristics, reproductive history, breastfeeding status, and family planning intention. Those who charted for 1 month were considered to be "learning" users from the first day charted. At 3-month intervals a follow-up form was completed to record any change in family planning intention and number of client/teacher contacts during the interval. Unplanned pregnancies were defined as conceptions to women who stated that they were using NFP to avoid pregnancy during the follow-up interval in which the pregnancy occurred. There were a total of 39 pregnancies in Liberia and

Table I. Relative risk for becoming an autonomous user in Liberia and Zambia (Cox hazards model)

	Liberia (612)	Zambia (955)
Age (yr)		
15-24*	1.00	1.00
25-34	1.00	0.85†
35+	1.07	0.97
Breastfeeding*		
No	1.00	1.00
Yes	0.80†	0.77‡
Housewife*		
No	1.00	1.00
Yes	0.81†	$0.64 \ddagger$
Rural*	1.00	1.00
Urban	1.00	0.96
Time period (of registration)		
1st 18 months*	1.00	1.00
2nd 18 months	1.44†	1.18
3rd 18 months	3.52‡	1.48
Visit intensity		
Low*	1.00	1.00
Medium	1.14	1.44†
High	1.23	3.52‡

180 in Zambia. Discontinuation also occurred for per-

sonal reasons. A total of 144 women in Liberia and 465

in Zambia discontinued use of the method because of

unplanned pregnancy or for personal reasons. Women

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whose intention changed from avoidance of pregnancy to planning a pregnancy did not stop charting NFP and were treated as censored at change of intention in this multivariate analysis. (For a discussion of women who

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^{*}Reference group.

 $[\]dagger p < 0.05$.

p < 0.001.

Table II. Relative risk for discontinuation of use of NFP in Liberia and Zambia (Cox hazards model)

	Liberia ($n = 144$)	Zambia (n = 465)
Age (yr)		3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
15-24*	1.00	1.00
25-34	0.94	0.87
35 +	0.99	0.56‡
Breastfeeding*		
No	1.00	1.00
Yes	0.14	0.55‡
Housewife*		
No	1.00	1.00
Yes	1.09	0.89
Rural*	1.00	1.00
Urban	7.50†	0.73
Time period (of		
registration)		
1st 18 months*	1.00	1.00
2nd 18 months	0.56†	0.79
3rd 18 months	0.39†	0.59‡
Visit intensity		
Low*	1.00	1.00
Medium	1.09	1.56†
High	0.58	0.39‡

^{*}Reference group.

changed their family planning intention see Kambic and Martin.2)

A learning user was classified as autonomous if she had learned the method and could chart her signs without further instruction. Autonomous clients were followed to ascertain their discontinuations and pregnancies. There were 612 autonomous users in Liberia and 955 in Zambia.

To examine categorical differences in discontinuation, life tables were calculated for 11 different socioeconomic, program, and client variables: (1) family planning intention (spacing vs limiting), (2) age, (3) parity, (4) education, (5) religion, (6) occupation, (7) place of residence, (8) breastfeeding status, (9) NFP method (i.e., either symptothermal or ovulation method), (10) intensity of teacher/client visits (three categories-low, medium, high), and (11) period of registration during the program (divided into the first, second, and third 18 months of the program).

A Cox proportional hazards model, which is a linear multivariate statistical model, was used to predict which of the above variables were significantly associated with autonomy, discontinuation, and unplanned pregnancy. The dependent variable is survival time for an event; for example time from the first day charged up to pregnancy, discontinuation, or autonomy. The independent variables are factors that are hypothesized to be related to the survival time until the event. The exponent of the coefficient of each independent variable provides

Table III. Relative risk for unplanned pregnancy in Liberia and Zambia (Cox hazards model)

	Liberia ($n = 39$)	Zambia (n = 180)
Age (yr)		
15-24*	1.00	1.00
25-34	0.80	0.83
35+	0.50	0.43§
Breastfeeding*		
No	1.00	1.00
Yes	0.71	0.28†
Housewife*		
No	1.00	1.00
Yes	1.19	0.99
Rural*	1.00	1.00
Urban	13.74†	0.69
Time period (of registration)		
1st 18 months*	1.00	1.00
2nd 18 months	0.93	1.38
3rd 18 months	0.44	0.68
Visit intensity		
Low*	1.00	1.00
Medium	2.28‡	0.93
High	1.14	0.44

^{*}Reference group.

an estimate of the relative risk of the event with respect to a baseline group; controls for the other variables in the model were applied. The Cox models were run with the SAS PHGLM procedure. The results of the Cox model agreed with the results of stratified life table analyses.

Results

Of the above variables, parity, education, and family planning intention were not found to be significant in either country in any model. These variables are not in the reduced models that are shown in Tables I, II, and III. In each table the reference group is indicated by an asterisk and has a relative risk of 1.0. The comparison groups have a risk for the outcome event that is calculated with reference to the reference group.

Table I shows the relative risk for becoming an autonomous user. In both countries, women who were breastfeeding and women who were housewives were significantly less likely to become autonomous users. Also, in both programs women who registered later in the program were more likely to become autonomous users, with the increase being significant in Liberia, and women who received more visits per month were more likely to become autonomous (significantly so in Zambia).

Table II shows the relative risk for discontinuation. Women who registered later in both programs were less likely to discontinue, and the lower risk is significant

 $[\]uparrow p < 0.01$.

p < 0.001.

p < 0.05.

 $[\]dagger p < 0.001$.

 $[\]pm p < 0.05$.

p < 0.01.

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in both countries. Visit intensity was significantly related to discontinuation of use in Zambia, and women who had more visits were less likely to discontinue. In Liberia urban women had a much higher risk (7.50, p < 0.001) than rural women of discontinuing. In Zambia women who were older than 35 and women who were breastfeeding were significantly less likely to discontinue use of the method. These associations were not seen in Liberia.

Table III shows the relative risk for unplanned pregnancy. In Zambia breastfeeding women have reduced risk (relative risk = 0.28, p < .001) of becoming pregnant compared with nonbreastfeeding women. In Liberia urban women had a 13.74 (p < 0.001) elevated risk of becoming pregnant compared with rural women. Also in Liberia women with a medium intensity of visits were 2.28 (p < 0.05) times more likely to become pregnant than women with the lowest intensity of visits.

Comment

Although several factors are associated with a higher probability of progression to autonomy or lower likelihood of discontinuation or pregnancy, no factors were found to be consistently associated with all measures of NFP use in both programs. The most consistent association was that women who entered the programs in the later time periods were more likely to become autonomous users and less likely to discontinue use of the method or to experience an accidental pregnancy. This might suggest that program improvements over time led to better NFP use. However, because of truncation, the results that relate to the time of registration should be interpreted with caution.

With the exception of unplanned pregnancy in Liberia, the results for visit intensity are as expected; more visits per time period resulted in a higher progression

to autonomy and less discontinuation and pregnancy. This suggests that program inputs can beneficially affect NFP use. In Liberia women with a medium visit intensity were more likely to become pregnant. It is possible that women who have unplanned pregnancies are more likely to become pregnant in the early months of NFP use when visits occur more often. However, since visits decline with time during learning, if women do not become pregnant, they may, on the average, have fewer visits per time period than women who have unplanned pregnancies and discontinued earlier.

Breastfeeding was significantly associated with better outcomes in Zambia but not in Liberia. This may relate to the differences in breastfeeding practices between the two countries. In Liberia urban women were at much higher risk for unplanned pregnancy (13.74) and discontinuation (7.50) but showed no increased risk for becoming autonomous. In contrast, the urban women in Zambia had a lower though not significant risk for discontinuation and pregnancy.

We conclude that it is difficult to predict autonomy, pregnancy, and discontinuation for African women who use NFP on the basis of socioeconomic variables. An often-used indicator for successful use, family planning intention, was never significant in explaining increased or reduced risk in this multivariate model. However, successful use of NFP was associated with possible program improvement over the life of the project and client visit intensity.

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Measuring natural family planning in terms of couple-years of protection

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Couple-years protection is a summary index of contraceptive protection. Couple-years protection allows comparisons of natural family planning with other methods. One couple-year protection for condoms is equal to 1 couple-year protection for pills and 1 couple-year protection for natural family planning. Critical in the calculation of couple-year protection for natural family planning is average duration of use. A working group on natural family planning recommended that 2 years of use be used as average duration until more data are available. (AM J OBSTET GYNECOL 1991;165:2063-5.)

Key words: Natural family planning; couple-years protection

What is couple-years protection?

Traditionally, family planning programs are evaluated with indicators such as numbers of users, client contacts, service outlets, and volume of contraceptives distributed. Evaluation measures like these, however useful, provide neither a summary picture of contraceptives dispensed nor a summary of the protection provided to clients through each type of method, much less through a mix of different methods. For example, how does one compare giving 10 condoms to one user and one cycle of oral contraceptive pills to another user?

The need for a simple summary index of contraceptive protection was successfully met by Samuel M. Wishik with the development of the couple-years protection (CYP) index. According to Wishik, the index is a composite measure of "person" (i.e., couple) and "time" (i.e., year) and, one could add, of "method" (i.e., protection).

One CYP means that one couple does not conceive for 1 year: this concept allows comparison of contraceptive methods. One CYP for condoms is equal to one CYP for the pill. A CYP is a standard measure of contraceptive protection.

The transition from the quantity of a given contraceptive method distributed to clients and the standard measure of protection provided by that contraceptive is accomplished by means of the conversion factor. The conversion factor is one of the two elements in the calculation of the CYP. The other element is the number of contraceptives provided to users.

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Definition and calculation of the conversion factor

The conversion factor is defined as "the average duration of protection provided by one application of the method." As an analogy, the conversion factor can be compared to the exchange rates that are used to convert different currencies into a standard currency. By transforming each quantity of a particular contraceptive into CYP, the conversion factor standardizes the protection that is provided by each method in terms of CYP. Not only can the CYP for one method be compared with the CYP for another method, but the CYP for all methods can also be added to summarize the total CYP achievements of a program.

For barrier methods (e.g., condoms and vaginal tablets), each unit of application protects the couple for one act of intercourse only. The value of the conversion factor therefore is inversely dependent on the coital frequency of the couples. Thus if the coital frequency of the couples was 100 times per year, the conversion factor would be higher than if the coital frequency was 144. According to the definition of the conversion factor in the first case, the calculation would give, 1/100 = 0.01, and in the second case, 1/144 = 0.007.

In the case of injectable contraceptives, in which the duration of protection of each unit depends on the duration of effectiveness of the dose, the conversion factor is independent of coital frequency. In this case one needs to know how many doses are needed to protect the client for a year. If the injectable contraceptive protects for 3 months, four units will be needed, and the calculation of the conversion factor would be 1/4 = 0.25. In the case of oral contraceptives, the same procedure applies except that instead of four units for the year, 13 are needed, since this is the number of cycles needed to protect the couple for 1 year.

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Table I. Natural family planning CYP achievement with different conversion factors

Type and number of acceptors		Conversion factor	CYP*
Example A	Lancaster		
Learners	177	0.9	159.3
Autonomous	265	5.7	1510.5
Total	442		1669.8
Example B	Working group		
Autonomous	265 1	2.0	530.0

*CYP = number of acceptors multiplied by the conversion factor.

For other semipermanent methods such as the intrauterine device (IUD) and Norplant, the conversion factor is independent of coital frequency and its value is directly related to the length of retention of the device in situ. The conversion factor for sterilization is based on the average age of the women who have been sterilized, subtracted from the age at menopause.

Calculation of the conversion factor for the natural family planning method

With the exception of NFP, there is a always tangible contraceptive unit (e.g., a cycle of pills, a condom, a diaphragm, a surgical procedure performed, etc.). For NFP, however, the effectiveness and continuation of use depends in great part on the quality of the teaching, on the clients' correct use of the method, and on the staff support that is given to clients. How do we know then what elements to include in the calculation of the conversion factor for this method?

Experience from the field indicates that there are two important elements to consider when the NFP conversion factor is calculated. The first is the distinction between the number of acceptors who are learners and the number of acceptors who are already autonomous. The second element is the duration of practice of the method by each type of acceptor.

The numbers of learners and autonomous users can be gathered from the program statistics. However, the calculation of the average duration of use requires long-term observation and follow-up of acceptors and clear definitions of the various events such as cases in which clients are lost to follow-up or drop-out for personal reasons or because of pregnancy, which can occur during the observation period. As with intrauterine devices and Norplant, life tables² or event calendars³ are used to determine duration of use.

Natural family planning acceptors: Learners and autonomous

According to the current literature on NFP, a learner is a person who registers in a program, attends instruction sessions, charts a certain number of cycles, and remains in the program for a given number of months. An autonomous acceptor is a person who has successfully completed the course of instruction and who is able to practice the method without assistance. In other words, the autonomous client correctly observes the signs of fertility and with her partner adjusts sexual behavior to achieve their family planning intention.

Observed values of duration of practice by acceptors of natural family planning

Much debate still exists about the average duration of protection that is conferred by NFP. Nevertheless, recent studies of good-quality NFP programs with strict definitions of discontinuation have achieved encouraging rates of continuation and effectiveness. For example, among the autonomous clients of the Saint Joseph's Hospital's NFP project in Lancaster, Pennsylvania, the average duration of practice of the method was found to be 5.7 years. For those who did not meet the requirements for autonomy, the average duration was only 0.9 years. More recently, Kambic and Martin found that the average length of use of "avoiders" was 3.10 years in Liberia and 2.72 years in Zambia, whereas for the learners, the durations were 0.71 and 1.12 years in these countries, respectively.

In the absence of special local studies, the recommendation of a working group that attended a seminar on cost-effectiveness of NFP and breastfeeding was "to assume two years of protection, based on the experience in the studies presented, until program data are available." The group also recommended that "costs and effects may be limited to the limiter/spacer proportion of the program."

Calculation of natural family planning CYP achievement

Table I shows that once data on the number of learners and autonomous acceptors and the average length of use are available, the calculation of the CYP achievement is quite simple. As indicated in Table I, it is done by multiplying the number of acceptors by the conversion factors.

Table I provides two examples of how to calculate a program's CYP achievement. In example A, the program has recruited 177 learner acceptors and 265 autonomous acceptors. By way of illustration, the conversion factors from the study in Lancaster, which was mentioned above, are used. The results indicate that, in this particular case the CYP achievement by learners was 159.3 and by the autonomous acceptors was 1510.5. Therefore the total CYP achievement in this program was 1669.8.

In example B, the program does not have figures of continuation for learners and autonomous acceptors; therefore, according to the recommendation of the

working group, the conversion factor of 2 years is aplied to the 265 autonomous acceptors. The result is 530 CYP or 57.7% less than in example A. If instead of the conversion factor for the Lancaster program one uses the conversion factor for Zambia, the results show CYP for learners of 198.3 and for autonomous acceptors, CYP of 720.8, for a total of 919.1 CYP.

In light of the above, it seems advisable that programs that offer NFP and meet the conditions below undertake the necessary studies on continuation of use of the method to calculate their own conversion factors. The conditions are:

- Both the population that is served by the program and the staff members who manage it know NFP well.
- · Special attention is given to the education and followup of acceptors. To do this, the institution maintains a good statistical system as well as a good network of communications and support for the clients (both for the acceptors and the spouses or partners) that is represented by counseling sessions and follow-up visits or calls.
- The medical and paramedical professionals who deal with the acceptors are sufficiently versed in the advantages and disadvantages of the NFP method and are also well disposed toward the clients.

• The clients are screened according to certain characteristics that predispose them to better acceptance and continuation of the method.

The decision on whether or not to undertake continuation studies and on whether or not to use the standard conversion factor of 2 years remains in the hands of program administrators and evaluators who know their programs.

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The modified mucus method in India

Kathleen Dorairaj, MD

Ghaziabad District, India

The modified mucus method—Prajanan Jagriti (fertility awakening) is intended to serve the cultural needs of illiterate and semiliterate women. Over 10 months, 3003 women in northern India were taught the modified mucus method. There were a total of 42 pregnancies in 24,702 cycles for a Pearl index of 2.04. High effectiveness is attributed to the support that was provided to clients by the instructor. (AM J OBSTET GYNECOL 1991;165:2066-7.)

Key words: Natural family planning; India; Prajanan Jagriti modified mucus method

In India, there is a need for a range of family planning methods to cater to cultural and sociodevelopmental differences in the country. The modified mucus method (MMM), referred to as Prajanan Jagriti in India, is intended to serve the cultural needs of illiterate and semiliterate women who have a low status in the family. It was developed to improve the acceptance of natural family planning (NFP) among nonacceptors of family planning, specifically poor women with low levels of intraspouse communication and whose husbands have little motivation for family planning.

The method is based on the woman's observation of cervical mucus and is similar to other mucus methods. However, there are some critical differences between the MMM and other more established NFP methods. Unlike other NFP methods, the MMM does not require a woman to chart her menstrual cycle. The MMM has a simplified set of rules for avoiding pregnancy and requires fewer days of abstinence per cycle than other NFP methods. It is believed that the simplicity and the reduction in abstinence will increase acceptability of the MMM.

Methods

The method is based on the woman's observation of cervical mucus, which she learns to do in a series of 10 home visits with the MMM teacher over three menstrual cycles. The visits are scheduled to coincide with the phases in the woman's cycle when she is most likely to need help with her observations and support in the use of the method. The rules of the MMM are less conservative than those of the ovulation method when pregnancy is not desired. The MMM allows sexual relations in the first month of use; the ovulation method does not. The MMM allows sexual relations on preovulatory days when thick or sticky mucus is present

From the Natural Family Planning Association of India. Reprint requests: Kathleen Dorairaj, MD, NFP Association of India, No. 31, Sector 37, Arunvihar, Noeda 201-301, Ghaziabad District, UP, India. 6/0/34201 in women who have regular cycles; the ovulation method does not. And the MMM allows sexual relations on the third day after the "feeling of wetness stops." The ovulation method rule is to wait until the fourth day after mucus peak. The greatest difference between the MMM and other NFP methods is that the MMM is seen as an entry point into the personal development process for poor, illiterate women. It does this by making women aware that they have power to control reproduction and by teaching them to use this power to better their lives.

Because the MMM is designed for use by illiterate women, it uses a structured approach to teaching and learning, and the timing and content of client visits are specified for the teacher. The structured approach to teaching the MMM and the supportive counseling that is given to the users may contribute to improved effectiveness compared with other NFP methods.²⁻¹

This study of 3003 acceptors was a part of the Training and Action Pilot Project for nonCatholics in North India, Uttar Pradesh, and Madhya Pradesh; the project covered 40 villages in six districts.

A training and action project was designed to train nonchurch, nongovernmental groups that are close to the grass roots to teach fertility awareness and NFP as an entry point to women's personal development. The project had a research component in which the acceptance and use-effectiveness in rural women was to be studied. The training project was used to mobilize women leaders by means of the infrastructure of small, grass-roots-level nongovernmental organizations. A systematic approach was used to select and train personnel including cluster coordinators and village leaders.

Results

In 10 months, 3003 menstruating women of proven fertility were taught fertility awareness and the use of the MMM. To ensure follow-up, teaching of the method was restricted and therefore the sample was contained. Only 10 new acceptors were taught each

month by each of the 37 village women leaders, who were supervised by 12 part-time cluster coordinators. All of the 3003 women completed the first 3 months of training. There were no unplanned pregnancies or drop-outs in the first month.

There were a total of 42 pregnancies in 24,702 cycles for a Pearl index of 2.04. The unplanned pregnancies occurred mainly in the women who had no living children and rarely in women who had one child.

The causes of unplanned pregnancy were considered to be:

- 1. The low motivation for family planning and the high psychologic need to prove fertility by having
- 2. The longer periods of fertility among young
- 3. The husband's difficulty in abstaining from sexual
- 4. The short duration of marriage and poor intraspouse communication.

The use-effectiveness of the method can be attributed to:

- 1. The motivational levels of the women/acceptors who had no alternative method available.
- 2. The teaching and support of the village women leaders.
- 3. The shorter periods of abstinence that were required because of the emphasis on the feeling of wetness.

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Discussion and recommendations

During the discussion period, some meeting participants expressed concern that extraordinary efforts were being made to do effectiveness and continuation studies when in fact operations research might be more useful. Some argued that the use-effectiveness of natural family planning (NFP) is established and that resources should be redirected to informing the biomedical and population community about NFP.

Table I in Kambic's article shows that a number of use-effectiveness studies already exist. However, both Kambic's and Labbok's articles pointed out some of the difficulties in attempting to generalize these results; for example, the broad range of pregnancy rates, the number of cultures and conditions under which the studies were done, and the advent of simplified modified mucus method NFP methods. Furthermore, use-effectiveness and continuation rates can be calculated in the context of operations research studies and surveys. In the large demonstration projects in Liberia and Zambia, Kambic and Gray used resources efficiently to describe a variety of outcome measures including effectiveness. Thus additional effectiveness studies in NFP will be

necessary, and they can be done concurrently with other studies.

A second issue raised by participants was disparity between family planning intention and behavior of some NFP couples. Why do couples who say they want to avoid pregnancy continue to have intercourse during the fertile time? Trussell and Grummer-Strawn have shown that these couples who break the rules, "risk takers," are much more likely to become pregnant than those who follow the rules. Frank-Herrmann found that a large proportion of German couples fall into this category. Their risk-taking behavior belies what these women and couples say about wanting to avoid pregnancy.

However, most NFP experts agree that having intercourse during the fertile time is not necessarily an expression of an intent to become pregnant. Sexual intercourse is often an emotional and not a logical act, and intercourse during the fertile time is not a reason to classify a pregnancy as planned unless the couple so indicates before the fact. Future research should focus on identifying couples who are likely to take risks and interventions to help them use NFP successfully.

6/0/34202

Abstracts*

Natural family planning training issues and strategies for the 1990s

Kimberly Aumack, Chair Mary Catherine Martin, Co-Chair Bonnie Pederson, Rapporteur

Introduction and overview of natural family planning training programs

Diane Vogelsang, BA

Quality natural family planning (NFP) instructor training is an essential component of NFP. There are several successful models for training NFP instructors. Synopses of four training programs are presented.

Competency-based approach to training and natural family planning instructor standards

Mary Catherine Martin, PhD

Competency-based natural family planning training programs are designed to prepare teachers to correctly demonstrate knowledge, supportive attitudes, and effective teaching skills.

An experiential approach to training natural family planning trainers

Kimberly Aumack, BA

To improve the quality of natural family planning (NFP) instructor training, the training division of the Institute worked with Western Consortium/International Health Programs to develop, conduct, and evaluate three international Training of Trainers programs. The purpose was to increase the NFP instructors' understanding of the training process, enhance their knowledge of NFP, and improve their skills in designing, conducting, and evaluating NFP instructor training courses. The training methodology used in these programs was based on current theories of adult education and experiential learning.

Preparing instructors to educate diverse client populations: The impact of culture, religion, socioeconomic status, and educational levels

Marie M. Mascarenhas, MD

Obstacles to widespread use of family planning in India include: limited access to services, cost, nonacceptability of many available methods, lack of information, and fear of side effects. Irrespective of caste or religion, most of the population practices different types of abstinence throughout their lives. There also are certain characteristics of successful natural family planning users in India. These factors should be included in natural family planning instructor training programs.

Training and supervision from small natural family planning programs to national service delivery systems

Sabina Mwaulu, RN

The Natural Family Planning Training and Medical Service Center in Nairobi, Kenya, conducts an instructors' training course for nurses employed by the Ministry of Health and other health and family planning organizations. The goal of the training is to develop natural family planning instructors who can provide high-quality services to their institutions' clients. Supervision after training is critical for program success.

Training evaluation: Its impact on future instructor training, client education, and natural family planning services

George Walter, MD

Training evaluation is a continuous process that includes needs assessment, monitoring, and evaluation of the immediate outcome of a course, as well as evaluation of the impact of training. The potential for multilevel evaluation to positively influence future natural family planning development in areas such as instructor training, client education, and service delivery is presented.

*The following abstracts of the nine sessions not contained in this JOURNAL are provided for your information. The nine sessions will be published, in their entirety, by Georgetown University in Spring 1992. For further information please contact Dianne Beekhuysen, Resource Center Coordinator, Institute for Reproductive Health, Georgetown University School of Medicine, Department of Obstetrics and Gynecology, 3800 Reservoir Rd., N.W., Washington, DC 20007; telephone (202) 687-1392.

Experiences and trends in natural family planning education and outreach

Rosalia Rodriguez-Garcia, Chair Leila Mehra, Co-Chair Earle Lawrence, Rapporteur

New directions for education and outreach in the 1990s

Rosalia Rodriguez-Garcia, MSc

Education and outreach are important to increasing awareness and use of natural family planning (NFP) and to reaching health professionals and policymakers. Two areas of intervention have emerged in the 1990s that could well make the difference between NFP remaining solely a method for a small and self-selected group of users or becoming a credible and widely used method: (1) empowerment of couples with fertility education and (2) use of sociodemographic data and sound communication strategies to increase the impact of NFP outreach programs.

Reaching new populations with natural family planning education

Leila Mehra, MD

Successful delivery of natural family planning (NFP) services is linked to education and understanding of the needs of special populations. Groups that are greatly in need of NFP services are identified: rural illiterate and underserved populations, young unmarried women and adolescents, and breastfeeding women.

Innovative approaches from population communication services: Implications for natural family planning

Benjamin V. Lozaré, PhD

To be effective, communication must be personal, popular, and persuasive. Entertainment-education is the key trend for mass communication as are computers for both interpersonal and interagency communication. Furthermore, entertainment for education must also be profitable.

Using quantitative data to define natural family planning use: A profile

Ravi K. Sharma, PhD

This paper addresses a twofold strategy to develop quantitative profiles of natural family planning (NFP) users: analyses of NFP service statistics and surveys that can provide retrospective information on NFP use.

Seeking opportunities for natural family planning expansion: The Serena experience

Suzanne Parenteau-Carreau, MD

The experience of the Serena natural family planning (NFP) program in Canada indicates that a multitargeted, comprehensive outreach program is very likely to succeed despite geographic distances and language differences. Outreach to decision makers and health professionals and direct advertising to reach concerned couples were key factors in the success of the Canadian NFP program.

Lessons learned from a mass-media campaign in Peru

Guillermo Tagliabue, MD

From June 1987 through June 1989, the main objectives of a recent study by the Asociacion de Trabajo Laico Familiar in Lima, Peru, were to: (1) improve dissemination of accurate and up-to-date information on responsible parenthood and natural family planning and (2) improve the training in natural family planning by means of specialized audio-visual materials.

Creating a demand for scientific natural family planning: The Zambian experience

Lubinda M. Tafira

There was very little demand for natural family planning before the Family Life Movement of Zambia began its 5-year demonstration program in 1981. However, the demand is higher today than the Family Life Movement of Zambia and its collaborating agencies can meet. This experience has taught us to be careful about creating a demand if there are insufficient resources to meet it. Today we are struggling with an expanding clientele with funds that have been cut by 60%.

Center for Research, Education, Service and Training's fertility education program for youth

Marie M. Mascarenhas, MD

Much pressure is exerted on India to control its population. To the illiterate although intelligent majority of Indians, our "biophilic" nature prevails—the love of family and life in the fields. Moreover, without the use of computers and statistics, rural couples have come to the correct understanding that to have three adult children, they must start with a family size of six children, because infant and child mortality alone takes a 40% toll, and with primitive methods of agriculture and fishing, a family needs three children to survive.

Teen STAR (Sexuality Teaching in the context of Adult Responsibility) year 4 cohort with one-year after program follow-up (United States) and report of Philippine cohort

Hanna Klaus, MD, and David Kardatzke, BSc

A study of 899 women and 308 men in the United States who participated in the 1988 to 1989 Teen STAR program indicates that the number of male and female subjects who engaged in sexual intercourse declined from entry to exit and 1 year after program. Similar but less significant results were obtained from a Philippine cohort. The results indicate that experiential learning of fertility delays teens' first sexual intercourse and reduces the number who continue to be sexually active.

Fertility education for young men

Stephen A. Burke, MSW

Young men are often overshadowed by the focus of fertility programs on women. What is taught, where, and who is taught, and who are the teachers are among the key factors that must be taken into consideration when planning fertility education for young men. For the sake of both young men and women, we need more balance in the content of fertility awareness programs provided to each gender. Young men's fertility signs, their strengths, insecurities, and role in relationships are often overlooked.

Training and educational materials

Lois A. Schaefer, Chair Margot L. Zimmerman, Co-Chair Ron Magarick, Rapporteur

Issues in the development and dissemination of training and educational materials

Lois A. Schaefer, MPH

As natural family planning begins to serve a more diverse clientele, appropriate education and training material are needed. Distribution of these materials, particularly those intended for worldwide or regional audiences, is a complex process. Planning for distribution should begin when materials are being developed.

Materials development, testing, and evaluation: An overview

Margot L. Zimmerman, BA, and Premila Bartlett, MPH

The Program for Appropriate Technology in Health's extensive experience in materials development is presented to illustrate the steps necessary to produce acceptable, relevant, and understandable materials. Three stages are given particular emphasis: the need for target audience reseach, involvement of members of the target audience in the development process, and ongoing pretesting and revision of materials.

Materials translation: Is that really what you want to say?

David Bowen, PhD, and Margareta Bowen, PhD

Translating written text or interpreting spoken material requires great care. Not only is knowledge of languages needed, but familiarity with the subject matter will also contribute to the quality of the final product. Professional translators are preferred, especially for major languages, because there are many difficulties inherent in the use of volunteers and native informants. When testing a translator's abilities, comprehension tests are preferable to back-translation.

Introduction of natural family planning information into multimethod materials for trainers

James Lea, PhD, and Catherine Murphy, MEd

The Program for International Training in Health materials for family planning trainers addresses a broad menu of contraceptive methods and a wide variety of service delivery settings, adapted to the country in which they will be used. Fertility awareness is taught as the basis for all methods; natural family planning is presented individually as one of the methods. Recognition of the need for specialized training in natural family planning and the broad service responsibilities of most health workers has led to an emphasis on a broad knowledge base and referral rather than on direct delivery skills.

Experiences in the field: Confederacao Nacional de Centros de Planejamento Natural da Familia, Brazil

Maria J. Sogayar, MAB

In Brazil, Confederacao Nacional de Centros de Planejamento Natural da Familia has developed a successful program by adapting its natural family planning teaching and materials to the audience. Materials are simple, with complex ideas of fertility illustrated by naturally occurring events that are familiar to users.

Materials development in Zambia: Concept versus reality

Lubinda M. Tafira

The Family Life Movement of Zambia's 5-year demonstration program to provide natural family planning services was the first of its type and scope in Africa. All the training materials were developed as the practical needs of the trainers, trainees, and users became evident. Today's elaborate text books and picturesque volumes were not available then; thus the Family Life Movement of Zambia began by developing its own materials. Later, the World Health Organization's Family Fertility Learning Resource Package was introduced and adapted to meet local needs.

The development of the International Federation for Family Life Promotion training guide

Mary Catherine Martin, PhD

In 1974 the Human Life Foundation with the Department of Health and Human Services, Office of Family Planning, and in collaboration with the International Federation for Family Life Promotion, began work on its document, *Instructor Training in Natural Family Planning (NFP)*. A task analysis of NFP programs and teachers in the United States and Canada was conducted. It revealed that teachers need to have sufficient and accurate knowledge and skills in fertility awareness to effectively teach ovulation and symptothermal methods to couples and provide follow-up services until couples reach autonomy.

The "Guide for Natural Family Planning Trainers"

Kimberly Aumack, BA

The ultimate goal of natural family planning instructor training and supervision is to ensure the provision of quality services. The likelihood of achieving this goal is greatly enhanced if the training courses designed for natural family planning instructors actually enable them to develop the knowledge, attitudes, and skills needed to effectively teach clients.

Service delivery part 1: Current programs and strategies for expansion

Gloria Mejia, Chair Antonio Solis, Co-Chair Richard Sevigny, Rapporteur

Materials in the United States: An example from Los Angeles

Carmen Minervini, BA

In the United States today, the natural family planning (NFP) provider serves people from diverse backgrounds. No longer is NFP solely the method for Catholics; it has become an issue of healthy birth control as well.

Client teaching materials for francophone Africa

Isabelle Ecochard, MD

A large portion of the population in francophone Africa has limited literacy skills and therefore requires highly visual and pictorial teaching aids. Materials for this population do not have to be complex to be effective, but to maximize comprehension and the effectiveness of natural family planning, they need to be easy to use and understand.

Materials for the modified mucus method

Kathleen Dorairaj, MD

In India, the potential acceptors of the modified mucus method have been identified as poor illiterate and semiliterate women living in remote villages. These women are nonacceptors of family planning because of low personal motivation, low motivation of the husband, or nonavailability and inaccessibility of family planning methods and services suited to their needs.

Current natural family planning programs and strategies for expanding service delivery: An introduction

Gloria Mejia, MD, and Antonio Solis, MD

Natural family planning (NFP) services are provided through an increasing variety of public and private sector programs, including community-based programs, Ministries of Health, family planning programs, religious-based programs, and others. To improve the availability of NFP services and increase the options for family planning clients in the 1990s, NFP programs must be offered through a variety of channels. The purpose of this session is to identify the gaps in current NFP service delivery and to summarize recommended strategies to expand NFP programs.

Natural family planning in a family planning program

Thomas Kring, BD/MTH

The Los Angeles Regional and the California Family Planning Councils oversee a network of state and federally funded programs through which more than 200 clinics in California provide free or low-cost family planning services. Included in the full range of services offered are natural family planning and fertility awareness. We believe that clients need to receive education about all methods, but the ultimate responsibility for method selection, as for all health care, rests with the individual.

New approaches to expanding natural family planning

David F. Skipp, BA

New approaches can be used to expand natural family planning service delivery and to develop more effective programs. To lower costs, offer higher quality services, and provide greater coverage for a "well-defined market," it is important to analyze existing natural family planning programs, research current operations, and test new approaches. This paper includes innovative ideas to use existing resources and to generate new or additional outside resources.

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Natural family planning: National program development in Kenya

Rose Wahome, RN, and Sabina Mwaulu, RN

In Kenya, the Catholic Church provides nearly one third of the total health services: close to 97% of these services include natural family planning (NFP). For the past 10 years, the Kenya Catholic Secretariat has opened more than 500 teaching centers, which include approximately 1300 volunteer NFP teachers and more than 74,000 registered NFP clients. More recently, NFP services have become available through public sector health services whose personnel are trained by the NFP Training and Medical Services Center.

Natural family planning through the health sector in India

Maria M. Mascarenhas, MD

Community health is a priority in India. The goal of the community health workers is to facilitate the emergence of a "self-energized family," of which fertility knowledge and natural family planning are an important part. Fertility awareness and natural family planning should be integrated into educational programs at all levels, along with other efforts to improve the health and status of women within both the family and the community.

Natural family planning through the health sector in Brazil

Maria J. Sogayar, MAB

In Brazil, legislative support for natural family planning (NFP) has increased the availability of NFP in the health sector. State and national laws ensure the provision of natural methods of family planning. As a result, responsibilities of the Confederação Nacional de Centros de Planejameto Natural da Família for planning and conducting NFP instructor training have expanded throughout the country. In addition, they provide NFP support and assistance to government and private organizations.

Expanding natural family planning through vocational schools for women in Cote d'Ivoire

Rosalia Rodriguez-Garcia, MSc

In Cote d'Ivoire, the Mouvement pour la Promotion de la Vie Familiale and the Ministry of the Promotion of Women introduced natural family planning (NFP) education into the national system of women's home economics centers and vocational schools. As of December 1990, 5000 women received fertility education, 837 were registered NFP users, and 389 were autonomous users. As a result of this project, a teaching module on fertility education and NFP has been officially integrated into the 2-year curriculum of the vocational schools of the Ministry of the Promotion of Women in Cote d'Ivoire.

An American national experience: Natural family planning services under the auspices of the Roman Catholic Church

Theresa Notare, MA

Natural family planning (NFP) services are offered under the auspices of the Roman Catholic Church in the United States to minister to married Catholics. The national office, the Diocesan Development Program for NFP, was formally established in 1981 by the National Conference of Catholic Bishops. Its mandate was to assist Catholic dioceses in increasing efforts to make NFP more widely known, respected, and used. A variety of projects enable the Diocesan Development Program to continue its work. There is an NFP coordinator or contact person in each of the 187 dioceses in the United States.

Service delivery part 2: Elements necessary for success

Victoria Jennings, Chair Shelagh O'Rourke, Co-Chair Richard Sevigny, Rapporteur

Introduction: Principles for meeting increased demand

Victoria Jennings, PhD, and Shelagh O'Rourke, PhD

It is expected that the demand for family planning will increase from 192 million users in 1990 to 286 million in 2000. The Agency for International Development has identified five principles on which family planning services must be based to meet this increasing demand. These principles, which apply to all family planning services including natural family planning, are that service delivery must (1) emphasize the quality of care; (2) expand to serve larger populations in more cost-effective ways; (3) evolve to accommodate a diverse, younger population and improved method mix; (4) include cooperation by all sectors, that is, government, private voluntary organizations and forprofit groups; and (5) be sustainable.

A management needs assessment for African natural family planning programs: Process and results

Darryl N. Pedersen, BA

Results of a management needs assessment of eight natural family planning programs in Africa indicate that these programs require technical assistance and training to improve their ability to analyze unmet need, quantify their objectives, evaluate the results of their efforts, and develop budgets and management plans linked to their objectives. Donors should streamline technical assistance to natural family planning programs. Program managers must be committed to improving their management practices.

Targeting quality of care: A strategy for successful periodic abstinence services

Zoe Kopp, MPH

Quality of care is an important concept in providing periodic abstinence whether services are provided by a natural family planning group or by a family planning group. Six elements of quality of care are relevant: (1) choice of methods to increase acceptability, (2) informing and counseling clients to improve effectiveness, (3) technical competence of providers, (4) personal relations between providers and clients, (5) mechanisms to encourage continuity, and (6) appropriateness and acceptability of services. Considering these factors can help programs provide women and couples with a safe, effective family planning method.

The effect of supervision on program development and quality of services

Richard St. Mart, BSc

Supervision is a vital link between program management and service delivery. It also links members of the organization into a team that provides high-quality, cost-effective services and efficiently achieves the organizations' goals. Through its supervision system, Action Familiale has improved service quality, decreased drop-outs and staff turnover, and decreased the amount of time for clients to reach autonomy in natural family planning use.

Automated management information systems

James Nesbitt, PhD

Programs should develop a sound manual management information system before they convert to a computerized system. Both manual and computerized systems should (1) be designed for specific management purposes, (2) collect data related to these purposes, and (3) focus on processes that influence the organization's ability to provide services effectively and efficiently. Commitment from top management to use and learn from the information generated by the system is crucial for success.

Adapting data systems of multimethod programs to incorporate natural family planning

Lilia I. Cuervo, MA

Including natural family planning (NFP) in data systems of multimethod family planning programs is an important step in increasing availability of NFP services. Among family planning associations in Latin America affiliated with The International Planned Parenthood Federation, the number of new NFP acceptors reported in program statistics increased significantly when family planning associations were encouraged to include this information and were given "credit" for providing NFP services. To incorporate NFP into a multimethod data system, family planning programs need to be shown the institutional, pro-

Cuervo (cont.)

grammatic, and political benefits of offering NFP. Reporting space for NFP should be provided in statistical forms, and the results of the programs' NFP efforts should be tabulated, analyzed, and publicized.

Budgeting and financial planning in natural family planning programs: In search of the perfect system

Darryl N. Pedersen, BA

Budgeting and financial planning are the central elements of good management. Although development programs must have technical commitment and merit to succeed, they must be well managed to have a significant, sustainable impact. Not-for-profit institutions must take their fiduciary responsibility seriously. The same budgeting and financial management tools that are applicable to other development efforts are applicable to natural family planning programs. Using these tools, managers can establish clear linkages between costs and resources, resources and activities, and activities and results. This is critical for all phases of the planning-implementation-evaluation process.

Policy issues in natural family planning

Victoria Jennings, Chair Lelia Mehra, Co-chair Paul Gross, Rapporteur

Gaining policy support for natural family planning

Victoria Jennings, PhD

Sustained success in any development effort requires support from relevant policymakers. Those who make decisions about resource allocation both in international donor and membership organizations and in specific countries and local institutions must (1) recognize the importance of a particular development effort and (2) take steps necessary to create an environment for its success. Natural family planning (NFP) is no exception. In fact, given many commonly held perceptions and the lack of accurate information about NFP, policymakers are often a barrier to the growth and availability of NFP services.

Natural family planning: Agency for International Development policy considerations

Sarah Clark, PhD and Jeffrey Spieler, MSc

The Agency for International Development support for natural family planning (NFP) is based on the principles of voluntary and informed choice. The Agency for International Development provides 5 to 6 million dollars annually for NFP and includes NFP in all areas of its programming, including research, information and training, service delivery, policy, and technical assistance.

Policies and support for natural family planning: Rationales and future plans

Jose Donayre, MD

The United Nations Population Fund supports natural family planning (NFP) as one of many important family planning options. There is a natural constituency for NFP, and NFP should be provided to meet their needs. Especially important is that the knowledge that NFP provides to women about their reproductive functions and the alternative reproductive and family formation behavior related to NFP empower women to exercise independence in setting their reproductive life course.

International Planned Parenthood Federation's policy and support for periodic abstinence: Rationale and future plans

Zoe Kopp, MPH

The International Planned Parenthood Federation acknowledges the importance of periodic abstinence-based family planning methods and encourages its members to make these methods available to increase family planning options. Couples who choose these methods should be informed of their advantages and disadvantages, including failure rates, and appropriate counseling and follow-up should be provided.

World Health Organization's policy considerations in natural family planning

Leila Mehra, MD

The World Health Organization (WHO) is continuing its leadership role in promoting and supporting the development of an integrated approach in maternal and child health and family planning programs, including natural family planning. Through its relationship with member countries, the WHO is taking specific actions to ensure that natural family planning information and services are available to those who choose to use them.

Understanding policy and the policymaking process: Considerations for initiating policy interest

Rosalia Rodriguez-Garcia, MSc

Initiating and sustaining policy action to support natural family planning are essential. Yet many policy-oriented efforts are unsuccessful because of the lack of understanding about what policy is and the context in which policymakers work. This article provides an overview of some of the considerations that are key to initiating policy interest for natural family planning.

Communicating with developing country policy-makers: The case for natural family planning

Elaine Murphy, PhD

Appropriate, effective communication with policymakers is essential for successfully introducing innovations, including natural family planning. Objectives must be clear, audiences must be identified, messages must be relevant to the audience, the source of the information must be appropriate, the channel of information must reach the desired audience, and the format must be compatible with the audience, the message, and the channel.

From policy to norms to services: Developing a consensus for natural family planning

David F. Skipp, BA

The climate for natural family planning (NFP) can be improved by working through the policy process, from official policy statements to the norms directing the actions of institutions responsible for implementing that policy, and finally down to the level of actual service delivery. All these levels must be addressed to ensure the availability of NFP as a family planning option.

XV. Roundtable discussions

Diane Vogelsang, Chair

The missing link—Why natural family planning is still "The best kept secret"

Kay Ek

This topic focused on the reasons why natural family planning has not been more successfully promoted and accepted by both the private and public sectors. Ways in which to present natural family planning in more positive ways were explored.

Making periodic abstinence more acceptable to natural family planning users

William Uricchio

Periodic abstinence has often been designated as one of the major barriers to broader acceptance of natural family planning. During this session, the group discussed ways to address the subject of periodic abstinence in a more positive light, stressing the benefits that can lead to strengthening the marital bonds, as well as enhancing the emotional and sexual aspects of marriage.

The fertility awareness method: Extent of use, potential, and research needs

Nancy Williamson

The fertility awareness method involves identification of the fertile period and the use of a temporary barrier method during the fertile phase rather than abstinence. Because this method is rarely taught in either natural family planning or family planning programs, there are no agreed on standardized instructions. As a result of this session, research priorities for the fertility awareness method were proposed.

Toward better marriages through natural family planning

Irene Osmund-Ruiz

The scientific basis of natural family planning (NFP), its effectiveness, and safety have been established. A new goal now is to establish the acceptability of NFP and its benefits in enhancing communication and understanding among married couples. Making known the positive aspects of NFP should help achieve this goal.

Providing natural family planning counseling within a family planning clinic

Carmen Minervini

In the past there has been concern that teaching natural family planning in a family planning clinic would be difficult. It was believed that a contraceptive mentality would undermine the philosophy that natural family planning is a way of life and that people working in a family planning clinic would not understand the need for abstinence.

A pilot study on teaching natural family planning in general practice

Cecilia Pyper

This discussion was opened with a brief description of a pilot study conducted in general practice in Oxford, England, from 1986 to 1988. The study confirmed that it was feasible for a nurse to teach natural family planning within a health care system and that group teaching was possible for some couples. The study showed a need for a well-structured course as well as audio-visual aids to reduce instruction time.

At what stage is natural family planning service delivery?

Hanna Klaus

For the past decade or so, publicly funded natural family planning programs have been at the level of demonstration projects. Funding agencies wished to have highly scientific, verifiable protocols and procedures to be able to justify awarding monies.

Klaus (cont.)

In the process, some believe that the delivery of natural family planning services has become secondary to the documentation of the delivery and effectiveness of the services.

Various forms of financial support for natural family planning teachers

Claude Lanctôt

In many countries around the world, natural family planning services, because of their essentially educational characteristics, have primarily been developed in nonclinical settings by a group of interested volunteers. There is a wide range of remuneration for natural family planning teachers, from the strictly volunteer to the fully salaried.

The promotion of exclusive breastfeeding and the lactational amenorrhea method in natural family planning programs

Kathy Kennedy

The purpose of this group discussion was to generate ideas about potential obstacles to the promotion and use of the lactational amenorrhea method through natural family planning programs and possible ways to overcome such obstacles.

Social science natural family planning issues

Ravi K. Sharma, Co-chair Jeffrey Spieler, Co-chair Nancy Williamson, Rapporteur Rochelle Shain, Rapporteur

Overview of social science and behavioral issues in natural family planning

Ravi K. Sharma, PhD, and Jeffrey Spieler, MSc

The number and characteristics of people who use natural methods is an important issue in natural family planning (NFP). National surveys of health and contraceptive use, such as the Demographic and Health Surveys and the Contraceptive Prevalence Surveys, provide some insight into this issue, particularly in countries in which reported use of periodic abstinence is high. From these surveys, the percentage and characteristics of women in a country or region who use (or have ever used) NFP can be determined and compared with users of other methods. Recent surveys also indicate the degree of accuracy of women's knowledge of their fertile and infertile days. Another important issue is a psychosocial factor that may affect choice, use, and continuation of NFP. This issue is difficult to study cross culturally but has significant implications for service delivery, teacher training, and counseling. This session addresses social science issues and proposes research questions that could investigate them more thoroughly.

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Knowledge and use of periodic abstinence

Martin Vaessen, PhD

The data on periodic abstinence collected by the Demographic and Health Surveys program include knowledge and use of periodic abstinence, use of modern contraceptives, method failure, and perceived problems associated with periodic abstinence.

Natural family planning use in Peru

Ravi K. Sharma, PhD

Natural family planning, which is used by a significant percentage of Peruvian couples, is discussed in terms of the sociodemographic characteristics of the respondents interviewed for the 1986 Demographic and Health Survey. Data show that natural family planning use and knowledge of the fertile period are positively related to women's education. Respondents also expressed concern about the perceived side effects of modern contraceptives.

The 1991 Mauritius contraceptive prevalence survey

Charles Chen, PhD, Jay Friedman, MPH, J. Shanker, BA, S. Kalasopaten, PhD, and Leo Morris, PhD

In 1985, Mauritius had an overall contraceptive prevalence rate of 75.3%, with 17.1% of currently married couples practicing natural family planning (NFP). Because this island nation has one of the highest prevalence rates of NFP among the developing countries, the 1991 Mauritius Contraceptive Prevalence Survey questionnaire will include a special module for both NFP and breastfeeding. Also, using a 5-year calendar, continuation rates and the use-effectiveness of NFP in this country will be evaluated. A review of results from the 1985 survey and analysis plans for the 1991 survey are discussed in this paper.

Psychosocial factors in natural family planning: An overview

Ravi K. Sharma, PhD

Based on existing research, psychosocial factors that influence the adoption, continuation, and effective use of natural family planning are summarized according to whether they are user, method, or context-related factors. Data are lacking on many of these variables. Many existing studies are methodologically and theoretically deficient.

Psychosexual aspects of natural family planning as revealed in the World Health Organization multicenter trial of the ovulation method and the New Zealand continuation study

Margaret M. France, MA

Psychosexual factors, particularly those related to abstinence, influence the effectiveness and acceptability of natural family planning. These factors have been examined by the World Health Organization multicenter study of the ovulation method and the New Zealand continuation study. Results of these studies are compared.

A profile of successful versus unsuccessful ovulation method users: Factors associated with unplanned pregnancy and nonadherence to the rules

Miriam H. Labbok, MD, MPH, Alfredo Pérez, MD, and Hanna Klaus, MD

This study identifies factors associated with the occurrence of unplanned pregnancy during use of the ovulation method of natural family planning (NFP). These include menses return, especially during breastfeeding, young age, and lack of previous experience with family planning. Whereas youth and lack of previous family planning are also associated with nonadherence to the rules, during the period of menses return, couples appear to be following NFP rules closely. These findings raise questions concerning the possibility of targeted counseling and special self-help groups, such as men's support groups, for subsets of the method acceptors. These findings also raise concerns about method rules during high-risk periods, such as lactational menses.

Operations research in natural family planning: Strategies, issues, and research approaches

Ed Ricci, Chair Myrna Seidman, Co-chair Mary Ann Sevick, Rapporteur

Current status of operations research in natural family planning

Ed Ricci, PhD, and Myrna Seidman, MPH

Although operations research (OR) has been widely used by family planning programs to improve program efficiency and acceptability, it has been applied minimally to natural family planning (NFP) programs. Although numerous factors inhibit the use of OR in NFP, the positive impact of these studies in family planning programs suggests that NFP should use OR to investigate issues of interest to the field.

Natural family planning program format effectiveness

Don Kramer, MST

This study compares the cost-efficiency and family planning outcomes of the Creighton Model of natural family planning and a team teaching format. Although the team teaching model was more cost-efficient, family planning outcome was identical in both models.

A pilot study on teaching natural family planning in general practice

Elizabeth M. Clubb, MD, Cecilia M. Pyper, MD, and Jane Knight, RN

This study examines the feasibility of teaching natural family planning in a general medical practice. Results showed high rates of use-effectiveness and relatively low cost. Additional benefits are helping couples with difficulty conceiving to achieve pregnancy, and assisting young women to better understand their own sexual development.

Evaluation of natural family planning programs in Liberia and Zambia

Robert T. Kambic, MSH, Ronald H. Gray, MD, Claude A. Lanctôt, MD, Mary Catherine Martin, PhD, Roselind Wesley, and Richard Cremins

A 5-year demonstration project was conducted to evaluate the use and cost-effectiveness of natural family planning in Liberia and Zambia. Pregnancy rates of 4.3% and 8.9% and costs of \$40.00 and \$30.00 per user were achieved in Liberia and Zambia, respectively.

Using operations research to improve natural family planning program services, management, and policy

Maria Wawer, MD, Theresa McGinn, MPH, and Regina McNamara, PhD

Operations research can be applied to natural family planning (NFP) programs. Results of operations research studies conducted in family planning settings can often be generalized to NFP. Because of the differences between NFP and other family planning methods, it is also important to identify particular problems of NFP programs and to develop and test solutions to address these problems.

Political and practical issues in the implementation of operations research studies in natural family planning

John Townsend, PhD, and Ricardo Vernon, PhD

Operations research (OR) has not been an important component of natural family planning (NFP) research. Most NFP services are provided by church-related institutions in which pastoral issues are more important than service expansion and effectiveness. Although NFP programs have limited resources and capability to conduct OR, these programs can learn much from the studies conducted by family planning programs. An OR study of NFP service delivery in Colombia is reviewed.